THE PURIN BODIES
OF
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And the Rôle of Uric Acid in Health and Disease

BY

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PREFACE TO SECOND EDITION.

To meet a demand, instead of a simple reprint, the first issue has been revised, several portions re-written, the results of recent investigations included, the literature brought up to date, and new estimations, etc., added. A chapter on the action of drugs upon purin excretion now appears, and an index and some tables of analytical methods are appended.

I have to express my thanks for the interest manifested in the first edition, and to apologise for the necessarily brief replies to many foreign and English correspondents.

Several blocks have been lent to me by the Editor of the "Practitioner," and it is a pleasure to here record my appreciation and thanks.

1903.

INTRODUCTION.

My object in undertaking the following investigation was to obtain further information as to the action of purin bodies and their metabolism, and to discover some means whereby the early pathological changes in certain metabolic disorders may be detected.

In endeavouring to solve these problems, I have made estimations of the purin bodies present in the more common foodstuffs, and studied their specific effects upon the metabolic processes in animals and man, when they are introduced into the body subcutaneously or taken by the mouth. Were it possible to recognise the first stages of the altered functions, the application of preventive and remedial measures would soon follow. The importance of the subject is emphasised by our knowledge of the secondary results of imperfectly katabolised substances upon the vascular and excretory systems. It has been aptly remarked that two-thirds of the cases of cardiac strain in the second half of life present a history of perverted metabolism.

I desire to specially express my indebtedness to Professor Stirling, Manchester, Geheimrath Hering and Professor Siegfried, Leipzig, Professors Johansson and Holmgren, Stockholm, for permission to work in their laboratories. My thanks are also due to them, as well as to Professor Santesson, Stockholm, and Dr. Burian, Leipzig, for their kindly interest and encouragement in an enquiry, which although by no means fulfilling the projected aims, will, I hope, furnish some basis for further work in a similar direction.

July, 1902.

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CHAPTER I.

The Chemistry and Physiology of Food Purins.

Since Kossel established the close chemical connection between nucleins and the xanthin bodies, many experiments have been made in order to determine the precise changes which occur during the metabolism of these substances in the animal organism. Few investigations, however, have been more pertinent and conclusive than the systematic enquiries which have been made into the sources of uric acid and which have ultimately differentiated the several factors in its formation. Of these factors the group named endogenous still compels the acknowledgement of its unknown origin, but the other, the exogenous, appears to be directly dependent upon the nucleins and xanthins contained in the ingesta, or in other words, upon the amount of purin bodies present in the daily diet.

The term "purin" has been applied by E. Fischer to a nucleus C_5N_4, and hence all bodies constructed upon such a base may be included under this name. The purin bodies of ordinary occurrence are *Hypoxanthin*, $C_5H_4N_4O$ 1—6 oxypurin, *Xanthin*, $C_5H_4N_4O_2$, 2—6 oxypurin, *Uric acid* $C_5H_4N_4O_3$, 3-oxypurin, *Guanin*, $C_5H_5N_5O$ 2 amino-purin, *Adenin*, $C_5H_5N_5$, 6—amino-purin, *Caffein* $C_5HN_4O_2(CH_3)_3$ tri-methyl-oxypurin, and *Theobromin*, $C_5H_2N_4O_2(CH_3)_2$, di-methyl-oxypurin.

Although current text-books treat the chemistry of the individual purins somewhat exhaustively, it is necessary to state some of the principal facts which underlie their group reactions in order to delineate the several phases of nuclein metabolism. The purin compounds crystallise easily, are more or less soluble in the usual solvents, and can now be oxidised and reduced. *Hypoxanthin* yields small crystalline scales with sharpened extremities almost like grains of wheat. *Xanthin* may be distinguished by its thin, flat, glistening rhombic plates, *Guanin* by small prismatic crystals or amorphous masses, *Adenin* by long needle-shaped prisms, and *Uric acid* by rhombic plates. Rarer forms have been demonstrated by variations in the media and rapidity of crystallisation.

Their solubilities present the following remarkable differences:—

	Hypoxanthin.	Xanthin.	Adenin.	Uric Acid.	Guanin.
Water: Cold	1:300	1:13000	1:1086	1:16000	Insoluble
Hot	1:78	1:1300	—	1:1600	„
Alkalies:					
Weak.	Soluble	Soluble	Soluble	Soluble	Slightly Soluble
Acids:	„	„	„	Insoluble	Soluble

By reduction of uric acid with chloroform in a sodium solution, *xanthin* first appears, and is followed by *hypoxanthin;* the latter finally splits up into CO_2, NH_3 and cyanamid bodies. By oxidation with ozone, cupric oxide, or potassium permanganate in neutral or alkaline solution, *uric acid* yields allantoin $C_4H_6N_4O_3$ and CO_2. On the addition of nitric acid,

or potassium chlorate and hydrochloric acid, uric acid breaks up into alloxan, $C_3H_2N_2O_4$ and urea, and by further oxidation, urea and oxalic acid are obtained, with parabanic acid, $C_3H_2N_2O_3$, and oxaluric acid, $C_3H_4N_2O_4$, as intermediate products. In ammoniacal copper solutions in the presence of air, uric acid yields oxalic acid and urea, and when mammalian blood is added to a solution of uric acid, and allowed to stand for several days at 20—35° C., the uric acid entirely disappears, and urea, oxalic acid, NH_3 and CO_2 are found to be present.

When nitrous acid is added to guanin ($C_5H_5N_5O$) xanthin is formed; adenin ($C_5H_5N_5$), plus nitrous acid, yields hypoxanthin, and by pancreatic digestion, hypoxanthin, xanthin and guanin are broken up into simpler bodies, but hypoxanthin is the least altered.

As regards their cleavage products:—

Uric Acid + HCl heated to 160°—170° C = glycocoll, NH_3 and CO_2.

Adenin + HCl heated to 180° C = glycocoll, NH_3 and CO_2 and formic acid.

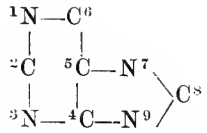
Xanthin + HCl heated to 200° C = glycocoll, NH_3 and CO_2 and formic acid.

Guanin + HCl warmed = glycocoll, NH_3 and CO_2 and formic acid.

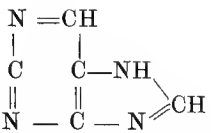
From a synthetic standpoint the purin bodies are exceedingly interesting. About twelve different combinations of the purin nucleus are known to exist in nature, but not less than 146 have been produced in the laboratory. Caffein and theobromin

are largely used as medicaments for their stimulative and diuretic properties, and it is possible that in the near future these may be made synthetically. Trichlorpurin, obtained by the action of phosphorous chloride upon uric acid, occupies a position midway between uric acid and the methyl xanthins, caffein, theobromin and theophyllin. Emil Fischer, in his lucid and interesting address given in Stockholm in November, 1902, after the distribution of the Nobel prizes, draws a picture of the time when the present coffee adulterants—chicory and coffee-surrogate—will be superseded by synthetically made caffein, and suggests a period when coffee beans and their roasting will be unnecessary, since the solution of a small powder in hot water will give a well-flavoured refreshing drink at a much lower cost and with much less trouble than the present conditions necessitate.

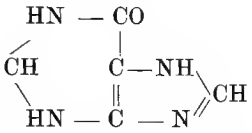
Through their affinity for silver and copper salts, the purin bodies may be fully precipitated by silver nitrate in ammoniacal solutions, or by cuprous oxide in the presence of sodium bisulphite. The separation of the several purins, although somewhat tedious, presents no special difficulties. The relation of these bodies to one another will be seen by their structural formulæ.



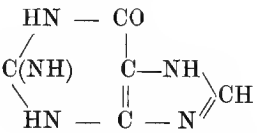
Purin nucleus.



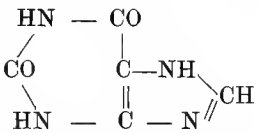
Purin.



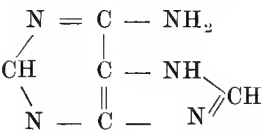
Hypoxanthin (Monoxy purin).



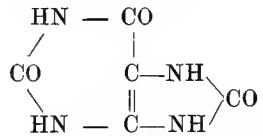
Guanin (2 amino purin).



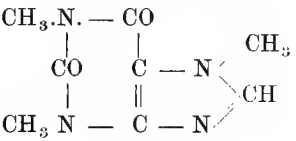
Xanthin (Dioxy purin).



Adenin (6 amino purin).



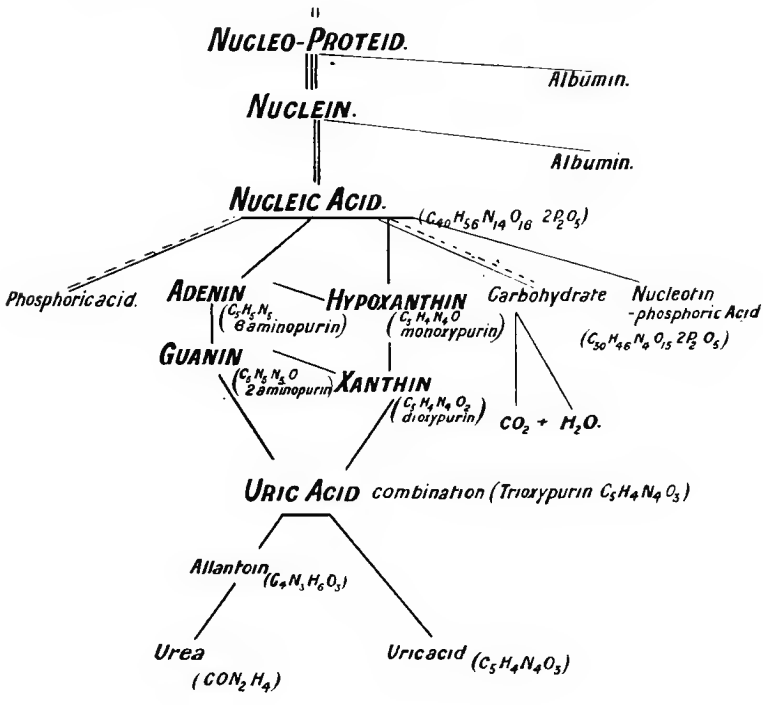
Uric acid (Trioxy purin).



Caffein.

From the above it would appear that purin consists of two urea radicles linked by a chain of carbon atoms, although it would be more correct to state that it is formed by the union of alloxan with one radicle of urea. Hence the term " alloxuric bodies " has been also applied to these substances.

The purin bodies exist either in a free state or combined with albumin in the form of nucleic acid. Hypoxanthin and xanthin occur in muscle extracts or are obtained by treating cell nucleins with H_2SO_4. Adenin is yielded chiefly by the decomposition of the nucleic acid present in thymus and guanin by the nuclein prepared from pancreas. The nucleins contain albumin and a carbohydrate (pentose or xylose) in addition to the nucleic acid. The following scheme shows the probable course of cleavage: —



The daily "wear and tear" or metabolism of cell constituents leads to the production of a certain amount of purin bodies. Whether this occurs in the vegetable, animal or human organism, these substances constitute the "endogenous" purins of the excreta. When tissues containing nucleins or free purins are eaten by lower animals or by man, the "endogenous" purins of the food ingested become "exogenous" to the system which absorbs them. As "endogenous" purins are practically waste products on their way to excretion, so when they become "exogenous" to another organism, they have little nutritive value and demand early and rapid elimination. This is generally effected by the oxidation of the oxy-purins, hypoxanthin and xanthin, to uric acid, and then the purin ring or chain in the uric acid is in the liver partially split off and a portion of the uric acid execreted as urea. The course followed in the case of the nucleins is not quite clear, as a smaller percentage appears in the urine as uric acid.

Amongst foodstuffs, with the exception of uric acid, the purin bodies are widely distributed. They exist in all forms of meat extracts, in the flesh meats of ordinary consumption, and in larger quantities in the glandular organs—thymus, pancreas, etc. In lesser amount they occur in many vegetables, as oats, potato, and sugar beet. Schultze and Bossard found them also present in the leaves and bark of certain trees, in young grass, and oat straw; in other plants they found a body *vernin* which, on the addition of HCL, yielded a guanin. Bethe states that the scales

of *Alburnus lucidus* consist largely of guanin. Griffiths found that the pigment of *Dienychylus viridescens* yielded uric acid when treated with HCl, and Hopkins observed that uric acid formed the basis of the white pigment of *Picrida brassica*. That the purins were first discovered in urinary calculi, and occur as regular constituents of human urine and fæces, are facts, which although well-known, may yet be cited, because they to a great extent express the chemical activity of individual metabolism, and the normal or abnormal conditions of the organic functions.

Before the connection between the purins and nucleins was known, the former were the cause of much controversy. Lehmann, Proust and many others investigated their effect upon the heart and circulation. Thudicum, in England, issued a treatise on the "origin, nature and uses of Liebig's extract" (1869), and the subject became one of distinctly polemic interest for both France and German scientists. Their conclusions attached little harmful influence to these bodies, but Gaucher, in 1886, observed degeneration of the convoluted tubule cells of the kidney after the injection of hypoxanthin, and von Noorden, in 1896, remarked upon their constant presence in the usual articles of diet, and the harmful influence their deficient excretion might exert upon the kidneys and general tissues. He considered it a matter for regret, however, that no data existed as to the purin contents of the various meats of common consumption, for quantitative determinations would have been of value in regard to the much-discussed

question of "white or dark meats"—veal, chicken, beef, mutton—in gout and kidney diseases, during general convalescence, and in disorders of the stomach and intestinal mucous membranes.

Senator and von Leyden state that the dark meats contain a larger amount of extractives than the lighter varieties, and hence are more irritating to the kidneys during excretion. Saundby, West, and Yeo condemn soups and meat extracts on account of their "extractives." Dyce Duckworth only allows the use of red meats once a day in nephritis; von Noorden permits their use in similar quantities to the white sorts, and Yeo prefers white meats alone; Dickinson advises light animal broths in nephritis; Luff is disinclined to their use in acute gouty attacks, but recommends either beef, mutton, chicken, turkey, pheasant or calf's sweetbread once a day during the intervals.

In order to obtain some tangible reasons for these opinions, Offer and Rosenqvist, in 1899, analysed certain meat foods, and were led to the conclusion that the variations in the amount of extractives contained in white and red meats were so slight that any theory as to their degree of irritative action, based upon such differences, could not be well founded. Their methods were not, however, free from objections, the varieties of foods they examined were but few, and even if their results were correct, it is obvious that the investigation calls for much more consideration than the simple analytical differences of the ingesta.

A deeper interest, however, centres in the specific

actions of food purins upon the various systems of the body, and their behaviour during the processes of metabolism. For such aspects are important in their relation to the construction of children's dietaries in connection with the evolution of the fermentative and chemical functions of their metabolic organs, and the tissue changes in certain diatheses. Not less significant are the action of purins in regard to the cessation of the processes of growth, the maintenance of adult life, and the gradual decline of bodily activities. Further, the *individuality* of "body" (Körper) chemistry is another factor which investigations upon these bodies has emphasised, and hence there is a need for some methods by which individual patients may be more accessible for the precise determination of their purin or nuclein metabolism.

The existent analyses of the purin-holding contents of food are best given in tabular form. They are but few in number. Hutchinson, in 1900, writes: "As regards the amount of extractives, but few data are available," and their absence from scientific literature has been frequently deplored. Jerome, in his admirable paper upon the relations between variations in the food and uric acid excretions, states that the alloxur-nitrogen of the food was not determined, and in the absence of precise knowledge as to the quantity of alloxur-nitrogen in both the food and fæces, certain deductions were impossible. Naturally, such estimations, together with those of the urinary purin, make too much demand upon the energy and time of one worker, but had any reliable

food purin analyses been then available, from Jerome we should probably have learned of the "endogenous" and "exogenous" factors of uric acid formation, instead of noting their appearance several years later in a language other than our own.

Table I.

The Earlier Estimations of Xanthins in Food.

	Hypoxanthin %	Xanthin %	Calculated as Purin Nitrogen %	
Beef	0·022		0·008	Strecker
	0·027		0·018	Neubauer
	0·016		0·006	
	0·156		0·062	Stadeler
Chicken	0·073		0·029	Kossel
	0·129		0·051	
Fowl		0·070	0·028	Hoffmann
			0·026	
Dove	0·037		0·014	Demant
	0·090		0·036	
	0·107		0·048	
	0·120		0·060	Kossel
Liver—ox	0·011		0·048	Stadeler
„ dog	0·082		0·033	Kossel
„ ox	0·053		0·021	

Table II.

Later Estimations of "Purins" in Food.

	% of purin nitrogen.	Calculated as purin %.	
Liver—dog ..	0·175 ...	0·4375	Kossel
,, calf ..	0·123 ...	0·3575	Burian und Schur
Beef	0·053 ...	0·1225	Burian und Schur
,, ...	0·030 ...	0·0700	Kossel
,, ...	0·046 ...	0·1150	Offer und Rosenqvist
,, steak...	0·071 ...	0·1775	,, ,, ,,
Veal	0·057 ...	0·1325	Burian und Schur
,, ...	0·030 ...	0·0750	Offer und Rosenqvist
Pork	0·031 ...	0·0775	,, ,, ,,
Mutton ...	0·034 ...	0·0850	,, ,, ,,
Ham	0·063 ...	0·1575	Burian und Schur
,, ...	0·052 ...	0·1300	Offer und Rosenqvist
Fowl	0·030 ...	0·0750	,, ,, ,,

Table III.

Comparison of the Later Estimations.

	Burian und Schur.		Offer und Rosenqvis	
	Purin N %	Purin %	Purin N %	Purin %
Beef ...	0·053 ...	0·1325 ...	0·046 ...	0·1150
Veal ...	0·057 ...	0·1425 ...	0·030 ...	0·0750
Ham ...	0·063 ...	0·1575 ...	0·052 ...	0·1300

Variations in the methods of extraction and estimation perhaps account for these marked differences. The quantities of purins found have

gradually increased from the earlier to the later investigators, but even were it possible to strike an average between the widely different figures of the later results, the analyses only include a few of the substances used as national food. Any estimations of such constituents should, however, be first approached from the standpoint whence the present results have diverged, so that a critical study of the methods employed becomes a necessary preliminary to the further consideration of the substances themselves.

CHAPTER II.

The Methods of Extraction and Estimation.

As the "purins" may exist in weak combination with certain proteid substances, or their precipitation be hindered by the presence of albuminous or phosphorus-holding bodies, one object of the methods employed has been to remove all proteids from the solution containing the purins, before attempting to obtain the latter as metallic compounds. The following papers contain the principal processes and modifications that have been used:—

Strecker, "'Liebig's' Annalen," 108, 1858.
Neubauer, "Zeit. für Analy. Chemie.," 16, 1867.
Salkowski, "'Virchow's' Archiv.," Bd. 50, S. 174, 1870.
Salomon, "Zeit. für Phys. Chemie.," Bd. 2, S. 65, 1879.
Demant, "Zeit. für Phys. Chemie.," Bd. 3, S. 387, 1879.
Kossel, "Zeit. für Phys. Chemie.," Bd. 7, 8, 1883.
Stadthagen, "'Virchow's' Archiv.," 109, S. 399, 1887.
Burian und Schur, "Zeit. für Phys. Chemie.," Bd. 23, S. 60, 1898.
Offer und Rosenqvist, "Berlin. klin. Woch.," S. 938, 1899.
Burian und Schur, "'Pflüger's' Archiv.," 80, S. 24, 1900.

The earlier of these workers extracted the minced-up substance with water at 50° C. for several hours, removed the proteids with lead acetate, and then pre-

cipitated the xanthins by silver salts, and crystallised them from strong nitric acid solutions. Kossel observed that extraction with a dilute solution of sulphuric acid accelerated the process and increased the yield, and so he boiled the organs in weak H_2SO_4 for some hours, then neutralised, removed the sulphate by baryta, the baryta by CO_2, and precipitated the purins by ammoniacal solution of silver nitrate. When, however, albumin is boiled for a long time in acid solution, small quantities of albumose are formed, and these enter into combination with the purin bodies, or in some way hinder their precipitation. Thus it became necessary to remove these from the solution. Kossel employed lead acetate for this purpose; Stadthagen used a double volume of 95 per cent. alcohol, as he found that with the lead acetate method $^1/_{26}$th of the total purin was lost. Burian and Schur, after removal of the albumins, precipitated the purins by ammoniacal silver nitrate solution in the presence of albumoses; from the filtrate they removed the silver by SH_2, dispelled the latter by heat, and precipitated the albumoses by a mixture of equal parts of basic and lead acetate, then re-precipitated with silver and added the amount of the resultant purin to that of the former. The length of this process and the rôle of the albumoses in preventing complete precipitation, provided the cause for my endeavours to overcome these difficulties.

In selecting precipitants, it was necessary to bear in mind the ultimate introduction of silver salts. Devoto's method for the separation of proteids by a

saturated solution of *ammonium sulphate* seemed worth a trial. The albumoses were not, however, entirely removed, and I found that the presence of $(NH_4)_2SO_4$ hindered the purin precipitation. For both these reasons, the method was inapplicable.

Tannic acid. It was not easy to obtain this reagent free from nitrogen, so control estimations were always necessary. To entirely remove it from the solutions before using the ammoniacal silver was also a matter of considerable difficulty. The precipitates produced, neither fell nor filtered easily, until the solution had stood for some days. An endeavour to hasten the process by the centrifuge was only partially successful. Although the remaining albumins were efficiently removed, the albumoses and gelatines were still suspended as flocculent flakes. An extract from boiled codfish, to which tannic acid was added on November 6th, 1900, after filtration, gave the following results by Kjeldahl's process:—

(1) After boiling in weak acetic acid solution and filtering, 0,0980 % N., estimated November 6th, 1900.

(2) After boiling in weak acetic acid solution and filtering 0,0910 % N., estimated November 6th, 1900.

(3) After adding tannic acid, 0,0908 %, estimated November 26th, 1900.

(4) After adding tannic acid, 0,0336 %, estimated June 20th, 1901.

Almén's solution of 4gm. tannic acid, 8cc. of 25 per cent. acetic acid, and 190cc. of 50 per cent. alcohol

was tried, but as the precipitates were soluble in excess of the reagent, it was not entirely satisfactory.

Stadthagen used two volumes of 95 per cent. alcohol, but even if this process were free from objections, the high government duty upon this scientific necessity renders the process too expensive for continuous use in England.

Zinc sulphate, according to Baumann, Bonner and Zuntz, precipitates albumoses as perfectly as saturated ammonium sulphate solution. They add 1cc. of 25 per cent. H_2SO_4 to each 50cc. of the albumose solution, and then saturate it with zinc sulphate. The precipitate contains NH_3, tyrosin, small amounts of kreatin and sometimes traces of leucin, in addition to that of the albumoses, but the flesh bases are said to be untouched. The method does not admit of easy application to the estimation of purins, as the presence of zinc certainly hinders their precipitation, and its hydroxide is not too easy to dissolve. An extract of raw codfish, which gave a good biuret reaction for albumose,

By ordinary methods yielded	0·2864	% purin N.
After $ZnSO_4$ precipitation yielded	0·1081	„
An extract of rabbits' muscles		
By ordinary methods yielded	0·1536	„
After $ZnSO_4$ ppt., removal of the Zinc by SH_2 and overplus of NH_4OH yielded	0·1523	„

The removal of the zinc by SH_2 appeared to render the method somewhat more precise, but its details were difficult to modify.

Beckmann's precipitation of proteids by *formaldehyde* was unfortunately quite inapplicable to the purpose in view. Krüger and Wulff's copper process precipitates other bodies than the purins, but yet, as many observers have shown, does not always completely precipitate the xanthins. Hence, although Malfatti found it reliable, it was not considered a safe method to employ. In the earlier days of the enquiry it was used as a control, but its variable results led to its discontinuance.

As gelatinous substances are present in extracts of cooked meats, it was necessary to provide for their removal. Salkowski found that flesh treated with water at a temperature not exceeding 30°C., yields no gelatine; but to obtain complete cleavage of the nucleins the minced meat must be boiled for some hours. The alcoholic method for the precipitation of gelatine is not now considered reliable, so that on the few occasions when a cold extract of meat was desired, the bromine process of Allen and Searle was employed.

The results of my experience gave so little satisfaction that I decided to proceed upon the lines of Burian and Schur's later method, modifying it as occasion arose. When I had used it for several months His and Hagen published a critical summary of the methods available for the estimation of purin bodies in animal organs. Their results led them to conclude that in the direct and correctur method of Burian and Schur, the first precipitate contained albumose as well as silver-purin, and hence gave a too high nitrogen result: that the albumose could

be removed by zinc sulphate or ammonium sulphate, but that purins were carried down with the albumose precipitate, and lessened the total amount of purin-nitrogen. This confirmed my own previous results. They also considered that no reliable method for the estimation of food purins existed, for the lead acetate method gave a loss, and although this was nearly constant in the case of beef, with rabbit flesh and other meats containing methylxanthins, the yield of nuclein substances was inconstant. It became imperative, therefore, to re-examine the method I had selected. Elsewhere I have stated the detailed results of such investigation made in conjunction with Dr. Burian (Zeit. f. Physiolog. Chemie., p. 336—396, Bd. 38) of which the following is a brief summary. From a critical standpoint it appeared necessary to answer the following questions:—

(1) Does the precipitate contain any nitrogenous bodies other than purins, and what is its precise chemical constitution?

(2) What conditions affect the total purin precipitation?

(3) What further modifications should be made?

To fully elucidate these points each step of the process was examined separately. (1) *Influence of the quantity of the solution and the length of the boiling.* The experimental results showed that the dilution of the solution, and the duration of boiling (above 12 hours) do not materially alter the total amount of purin obtained, but that in dilute solutions the first precipitate is small, and the second or "correctur" precipitate is larger, and if the solutions

are evaporated down to 200—300cc. before the ammoniacal silver nitrate is added, the "correctur" precipitate is so small that it may be almost neglected.

The addition of barium hydrate solution. The figures obtained from estimations of glandular and muscular tissues show that when the original solution is made weakly alkaline by barium hydrate, more albumose is present after filtration than when it is made strongly alkaline; hence after weak barium alkalinity there is a greater hindrance to the purin precipitation, and the "correctur" or second precipitate contains the greater proportion of the purin. Additionally, with a marked surplus of $Ba(OH)_2$, the introduction of CO_2 produces its more complete removal.

The evaporation of the solution before silver precipitation. Comparative estimations of the purin-contents of solutions evaporated in neutral and acid media, showed that unless marked acidity is maintained, upwards of 50 per cent. of the total purins are decomposed and lost. When the solutions were very concentrated, it was found that some of the less soluble purins formed small insoluble masses, whose solution necessitated the addition of sodium hydrate and sodium carbonate for the removal of any remaining barium compounds.

Influence of washing upon the silver-purin precipitate. Very large amounts of pancreas, thymus and beef were taken, the obtained purin precipitates dried in vacuo, then decomposed by HCl and the resultant filtrate tested for the presence

of albumoses. When the precipitate had been washed with *cold* water, a distinct biuret reaction was obtained, but if water at 60°C. had been employed, the biuret reaction was absent or of very faint colour. The same result appeared in quantitative estimations. If the precipitate from a peptone solution containing a known quantity of guanin was washed with cold water the purin nitrogen was always too high, but after hot washing the figures approximated the nitrogen of the added guanin and were entirely satisfactory:—

Peptone Solution. 6%	Guanin N added.	Guanin N recovered. Hot washing.	Cold washing.
20 cc. in 200 cc.	0·0244	0·0228	0·0232
20 cc. in 340 cc.	0·0644	0·0604	0·0663
20 cc. in 640 cc.	0·0545	0·0561	0·0616
20 cc. in 640 cc.	0·0545	0·0564	0·0605

The examination of the precipitate itself. The washed precipitate was subjected to elementary analysis. The relation of C – N in hypoxanthin and xanthin is as 15 : 14, and in guanin and adenin as 12 – 14.

Estimations of Sheep's Pancras.

Found		Calculated	
C	= 19·33%	C	= 15·66%
H	= 1·56%	H	= 1·30%
N	= 22·67%	N	= 18·27%
Ag	= 49·46%	Ag	= 56·40%

With the presence of ammonia and the repeated washing, the possibility exists that a small amount of silver oxide may be formed, but the proportions of C – N in guanin are 12 – 14, and in this case are as 11·91 : 14.

With *thymus*,

C = 24·55.
N = 17·50. ∴ C − N = 19·6 : 14.
Instead of C − N = 15 : 14.

Another estimation of *thymus*

C, 20·15.
N, 11·55. ∴ C − N = 24·4 : 14.
instead of C − N = 15 : 14.

In *muscle*,

C = 23·10
N = 14·23, hence C − N = 22·9 : 14, instead of 15 : 14.

The precipitate from pancreas thus appeared to be quite pure, but those obtained from thymus and muscle yielded too high a percentage of C, although the amount of N was correct. As in these estimations the nitrogen percentage is all that is necessary, the precipitates are therefore sufficiently pure for practical purposes. In order, however, to remove the surplus carbon, extracts of horseflesh were precipitated by phosphotungstic acid. After filtration, the solution contained a small amount of unprecipitated purins. The excess phosphotungstic acid and the H_2SO_4 were removed by $Ba(OH)_2$, and the barium precipitated by CO_2. The solution, after being entirely freed from any remaining traces of barium by the addition of ammonium carbonate, was evaporated and dried at 100°C. until constant, then weighed and decomposed. It yielded the following results: —

C = 19·35%.
H = 1·40%.
N = 18·12%.
C : N = 15·5 : 14.

As the calculated proportions of C : N in xanthin and hypoxanthin are 15 : 14, the precipitate was entirely pure, and thus allowed accurate results to be obtained.

Quantitative results. The figures quoted in regard to the washing of the precipitate show that the guanin added to a peptone solution can be almost completely recovered by this process. The following results additionally confirm the conclusion that the method is thoroughly reliable.

Found. Purin.	Purin substance added.		Total Purin. Calculated.	Total Purin. Found.	Percentage difference.
		Muscle Extracts.			
0·0551	Xanthin	0·0261	0·0812	0·0821	+1·1%
0·0620	Guanin	0·0777	0·1397	0·1421	+1·7%
0·0620	Guanin	0·0777	0·1397	0·1510	+8·1%
0·0713	Guanin	0·0259	0·0972	0·0991	+1·9%
0·0713	Guanin	0·0259	0·0972	0·0907	−0·2%
0·0629	Hypoxanthin	0·0262	0·0891	0·0928	+4·1%
0·0310	Hypoxanthin	0·0229	0·0539	0·0526	−2·4%
		Thymus Extracts.			
0·1568	Guanin	0·0726	0·2294	0·2287	−0·3%
0·4240	Guanin	0·0259	0·4499	0·4496	−0·1%
0·4240	Guanin	0·0518	0.4758	0·4818	+1·2%
		Pancreas Extracts.			
0·0525	Guanin	0·0363	0·0888	0·0877	−1·3%

The modifications which appeared to hasten and facilitate the process are, perhaps, best included in a detailed description of the method.

1. *Extraction.* The well-hashed meat was boiled for twelve hours in 10—20 volumes of 0·5 to 1 per

cent. sulphuric acid. After filtration the residue was boiled several times, filtered and well washed with at first acidulated and later with distilled water.

2. *Preparation of the extract for the "direct" precipitation.* The filtrate from 1 was saturated with powdered barium hydrate to strong alkaline reaction. After filtration the barium precipitate was well washed with water at 60°C. and CO_2 passed through the filtrate until the fluid became neutral or slightly acid. The carbonate of barium precipitate was then removed, and after thorough washing, acetic acid added to the filtrate, and the latter evaporated to 100cc. for each 100gm. of the organic material. It was then made alkaline by the addition of a few cubic centimetres of a mixture containing equal volumes of 33 per cent. NaOH and half-saturated sodium carbonate solution. Any resultant precipitate of $BaCO_3$ was then removed and the filtrate first made acid with a few drops of strong HCl and then saturated with ammonia solution.

3. *The direct precipitation.* To the filtrate from 2, not exceeding in quantity 200cc. for each 100gm. of meat, 30–50cc. of Ludwig's ammoniacal silver nitrate or chloride solution was added. The precipitate obtained was first washed with very weak ammonia, then with hot water until the washings were neutral; afterwards boiled with a little magnesia (after Arnstein's method) and its nitrogen then estimated by Kjeldahl's process.

4. *The indirect or "correctur"* precipitation. The filtrate from 3 was acidified with glacial acetic acid, the silver decomposed by H_2S, the residue boiled

several times with weak acid and filtered, the H_2S driven off by heat, and the fluid evaporated to 100cc. for each 100cc. of meat. Basic lead acetate was then added until the solution was alkaline and precipitation hastened by the addition of a small quantity of talc. The residue was well washed with cold water. After removal of the lead by H_2S or H_2SO_4, the filtrate therefrom was evaporated in acid solution to 20 – 30cc. for each 100gm. of tissue, and ammonia and ammoniacal silver solution added. The silver-purin precipitate obtained was treated as in 3, and its nitrogen added to that obtained by the direct precipitate. The amount of the two results gave the total purin.

The old lead-acetate method was used as "control" on several occasions, but always showed a slight loss as compared with the direct method. At this point the process adopted by Offer and Rosenqvist may be discussed. They took 25 – 40gms. of hashed flesh, added 250cc. water, and allowed it to stand upon ice for 12 – 20 hours. The proteids were removed by boiling during three periods of 15 minutes each, when albumin tests were said to be entirely negative. It was deemed unnecessary to acidulate, as the flesh itself was slightly acid. After the filtrate had been made up to 500cc., 100cc. (5 – 8gms.) were taken for the estimation of the xanthin bodies. The method was simple and expeditious, but could scarcely yield more than approximate results. The ice extracts would certainly contain the free "purins," but the bound "purins," which consist of 15 to 25 per cent. of the total amount, would be unextracted.

Further, in regard to the removal of the albuminous bodies, if the reaction of the filtrate was sufficiently acid to produce complete coagulation, it would probably, during the 45 minutes at 100°C., cause the formation of small quantities of albuminates or albumoses. On the other hand, Fürth has shown that plasma obtained from the muscles of fishes contains a proteid, myoproteid, which is not coagulated by heat, and is only precipitated by strong solutions of acetic acid; this by Offer and Rosenqvist's method would remain in solution, swell the total extractive N and prevent the complete precipitation of the purins. There is no mention of filtration after the addition of NH_4OH, so that while the purin-N would be too small from the imperfect extraction, the presence of other nitrogenous bodies would incorrectly increase its amount. I used Rosenqvist's method in an estimation of chicken flesh, and obtained 0·0262 purin-N. This compared favourably with Offer's results 0 0300 purin-N. The *residue* from the cold extract was then boiled with 0·5 per cent. H_2SO_4 for 12 hours, and precipitated with $Ba(OH)_2$, the $Ba(OH)_2$ removed by H_2SO_4, and the filtrate precipitated with $AgNO_3$ and NH_4OH. The precipitate yielded 0·0144 purin-N. The *filtrate* from the first silver precipitate was acidified, the silver removed by H_2S, the filtrate heated until the H_2S was dispelled and then precipitated with a solution of subacetate of lead. After removal of the lead, $AgNO_3$ and NH_4OH were added, and the precipitate obtained yielded 0·0087 purin-N.

Thus the original result of 0·0262 N. by Offer's method
plus ... ... 0·0087 N. from „ filtrate
plus ... ... 0·0144 N. „ „ residue

gives a total of ... 0·0493 purin N.
Estimations by the method as described above gave
0·0501 purin N.
0·0532 „ „
so that there was a loss of 0·023 N. or 0·055 xanthin bodies by Offer and Rosenqvist's method (0·0493—0·0262).

For purposes of comparison, the total nitrogen and the total "extractive" nitrogen were also estimated; 1–2gm. were taken for the former, and 25–50gms. for the latter. A cold extract was made, and the N. taken after the removal of the albumins and albuminates. The result was naturally only approximate, as the bound nucleins were unextracted, and a small amount of albumose may have been present. The precise total quantity was not, however, pertinent to the enquiry.

In relation to the effect of food purins upon the urinary excretion, it was necessary to examine the *total urinary purins*. For their amount, Arnstein's modification of Camerer's method was employed.

Uric acid was estimated by Hopkin's ammonium chloride process, and the *xanthins* calculated by difference. In the former case the crystals were weighed, and control studies were made by the Ludwig-Salkowski process. *Phosphoric acid* was titrated against uranium nitrate and expressed in terms of P_2O_5.

For the extraction and estimation of purins in

vegetables and beverages, 250gms.—2 kilos were boiled for twelve hours in 0·5 per cent. H_2SO_4 solution. This was then neutralised, and the filtrate therefrom was acidified with acetic acid and again filtered. The extract was next boiled for 5—10 minutes and any resultant precipitate removed. Excess of copper sulphate and sodium bisulphite were then added, and the fluid again boiled. After standing for 2—10 hours the precipitate was filtered off, and the residue well washed with water at 60°C. I found it of advantage to allow the precipitate to dry until cracks appeared, and then grind it in a mortar until it was entirely pulverised. This facilitated the next step of suspension in boiling water, and the subsequent precipitation of the Cu as copper sulphide by H_2S or K_2S. This precipitate was washed with H_2S water, the filtrate freed from H_2S by heat, and evaporated to about 300cc. After the addition of NaOH and Na_2CO_3, subsequent filtration and acidification, strong ammonia solution was added. When the fluid gave no proteid reactions silver nitrate was used, and the purin-N. found by Kjeldahl's process as usual. This method gives satisfactory results, except when large quantities of proteid matter are present, as in the legumes. With these it appeared safer to take the final filtrate and treat it in the same manner as that from the meat extracts, adding the two results together. As a rule the copper precipitation was repeated twice or thrice, until the silver purin precipitate was colourless.

CHAPTER III.

Estimations.

The Quantities of Purins in Meat Foods.

In mammalian muscles a considerable quantity of fat is found, and by its presence in streaks within the lean portions of meat, the quality of a specific cut or joint is more or less judged. The object of the breeder is, therefore, to produce in his cattle the appearances which the public prefer during the several seasons, and he obtains these by variations in the feed and environment. As the percentage of fat is thus changed according to the time of the year, and may vary between 1·5—29 per cent., the quantities of the other constituents will be proportionately altered. Still, analytical results show certain limits of variation in regard to the different species, and so permit of their application to scientific and clinical purposes. Additionally, the many parts of the same animal vary both in regard to their proteid and extractive percentages, and this fact should be also remembered. With fish and fowl flesh, these matters are of less moment.

The following table is constructed from my own estimations, and is intended to show the amounts of purin bodies contained in the more common of

the meats found in the English dietary. The extracts were made from flesh purchased in the ordinary way from a butcher, and with the fat and other contents intact, just as they are used in the household. This was thought preferable to the separate analysis of lean and fat portions, which would have made the results inapplicable to the necessities of daily practice.

TABLE IV.

	Percentage of Purin Nitrogen.	Average % of Nitrogen.	Calculated as Purin bodies.	Undried Purins. As grams per kilo.	Undried Purins. As grains per lb.
FISH:					
Cod	0·0219	0·0233	0·0582	0·582	4·074
	0·0247				
Plaice	0·0334	0·0318	0·0795	0·795	5·565
	0·0302				
Halibut ...	0·0405	0·0408	0·1020	1·020	7·140
	0·0412				
Salmon ...	0·0482	0·0466	0·1165	1·165	8·155
	0·0450				
MEATS:					
Tripe	0·0235	0·0229	0·0572	0·572	4·007
	0·0224				
Mutton—					
Australian	0·0365	0·0386	0·0965	0·965	6·755
English ...	0·0411				
Veal—					
Loin ...	0·0454	0·0465	0·1162	1·162	8·137
	0·0481				
Neck ...	0·0300				
Pork—					
Loin ...	0·0485	0·0485	0·1212	1·212	8·487
Neck ...	0·0257	0·0227	0·0567	0·567	3·969
	0·0198				

Ham	0·0505	0·0462	0·1155	1·155	8·085
*(Fat) ...	0·0419				
Beef—					
Ribs ...	0·0455	0·0455	0·1137	1·137	7·959
Sirloin ...	0·0506	0·0522	0·1305	1·305	9·135
	0·0538				
Steak ...	0·0826	0·0826	0·2065	2·066	14·455
Liver	0·1125	0·1101	0·2752	2·752	19·264
	0·1078				
Sweetbread—					
Thymus ...	0·4025	0·4025	1·0063	10·063	70·431
Chicken ...	0.0495				
	0·0546	0·0518	0·1295	1·295	9·065
	0·0512				
Turkey ...	0·0504	0·0504	0·1260	1·260	8·820
Rabbit ...	0·0305	0·0380	0·0970	0·970	6·314
	0·0456				

These figures call for little discussion at the moment. Certain meats appear richer in purin than others, but with the exception of liver and sweetbread, and when the amount of each sort necessary to provide the requisite amount of proteid or the feeling of satisfaction, is calculated, there is not much difference between the several species. More fish is generally eaten than beefsteak, if the meal consists solely of one or the other; tripe is usually a supper luxury, and sweetbread is, as a rule, taken sparsely.

But the form in which the purins occur is interesting in regard to their metabolism, and further investigations were undertaken in order to

*Considerable difficulties were experienced with this owing to the use of curing substances, principally potassium nitrate.

determine the relations of the free and bound purins in meat foods. For this purpose an extract was made with 0·5 per cent. H_2SO_4 solution, and kept for 24 hours upon ice, as Meischer has shown that there is no cleavage of nucleins when the temperature does not exceed 2—3°C. The filtrate from this extract contained the free purins and was treated as usual. The residue was boiled for 12 hours in 0·5 per cent. H_2SO_4, and then precipitated by a mixture of basic and lead acetate, the lead removed by H_2SO_4 and the purins obtained as silver compounds. It is possible that a small quantity of the bound purin was present in the first filtrate, but it may be safely neglected. Appended to the table stating the results obtained are the figures of Burian and Schur and of Katz showing the relation of the phosphorus and sulphur to the bound purin.

TABLE V.

THE "FREE" AND "BOUND" PURINS OF MEATS.

	"*Free*" purin. Nitrogen.	"*Bound*" purin. Nitrogen.	P_2O_5.	Sulphur.
Cod	0·0299	0·0106	...	...
Tripe	0·0143	0·0092	...	...
Chicken ...	0·0348	0·0147	...	...
Ham	0·0398	0·0064	4·8702	2·0430
Oxflesh ...	0·0460	0·0070	3·8944	1·8677
Veal	0·0430	0·0100	5·0291	2·2586
Liver	0·0330	0·0790	...	...
Sweetbread	0·0420	0·3510	...	...

As possessing some interest in relation to the percentage of purins already cited, and as a con-

tribution to existent analyses, my estimations of the total nitrogen, and the total extractive nitrogen of the meats examined are given in the following table:—

TABLE VI.

	Total N. %	Total Extractive N. %		Total N. %	Total Extractive N. %
Cod... ...	4·445	0·2659	Pork ...	2·576	0·2097
	4·645	0·1949		2·324	0·2151
	4·922			2·216	0·2276
Plaice ...	3·356	0·5600		2·284	
	3·040		Ham ...	4·200	
Halibut ...	3·370	0·3570	*dry* ...	5·840	0·4710
	3·360		...	5·860	
——			Beefsteak	4·900	0·4800
Tripe ...	2·800	0·0980		4·390	
	2·600	0·1036	Chicken...	3·560	—
Mutton ...	3·102	0·3908	Rabbit ...	3·245	0·2450
Veal ...	3·136	0·4690		3·382	
	2·856	0·3250	Liver ...	3·122	0·2850
				2·904	

The amount of total nitrogen multiplied by the factor 6·25 will give the proteid percentage. The extractive nitrogen consists of creatin, creatinin, hypoxanthin, xanthin, in some instances methyl-xanthins, and perhaps urea, carnin, and inosinic acid.

Considerable emphasis has been laid upon the alterations produced in the amount of extractives by cooking, and one infers from the remarks of Senator that the various kinds of meat may yield altered percentages of residual purins when prepared and ready for eating. Apart, however, from the fact that both roasting and boiling render meat more

sapient by coating its surface with extractive matter, supplying a solution of purins, etc., known as gravy, and lessening its digestibility by the coagulation of proteids, there is only a very slight decrease in the amount of total purins.

If it were customary to eat meat food, minus the gravy produced during the process of preparation, the matter might be of moment, but as not only the gravy obtained from the meat, but also an additional purin contribution from some fashionable meat extract is served, it is probable that more purin is taken with the cooked meat than exists in the freshly killed muscles. Hence, although the percentages of purin nitrogen are expressed in terms of the raw undried substances, they may be satisfactorily applied to the several forms in which animal food is usually partaken.

The Purins of Vegetable Foods.

It is usually stated that while vegetables contain much water, indigestible cellulose and varying amounts of proteids, their extractive matter is very small. Consequently only a few stray estimations are recorded of the purins existent in vegetable foods. Jerome noticed an increased uric acid excretion after the ingestion of large quantities of large quantities of asparagus, and was led to investigate its nuclein percentage, and found 0·0854gm. in 500gms. of asparagus (0·0171 per cent.). The Krüger-Wulff method was employed. Burian and Schur with the object of finding a purin-free food, analysed

bread, potatoes, rice and cabbage, but observed traces of purin bodies. The following determinations will not only confirm the general impressions but furnish precise data in regard to the nuclein nitrogen of vegetable life. The percentages are certainly small, but if vegetable food is solely used, larger quantities are required than when meat is taken. As a rule, plants have no excrêta except gaseous bodies, so that katabolic resultants of a solid nature are retained within the organism; these excretory products are, however, always removed to such localities as ensure their withdrawal from the spheres of vital activity, and are met with in not inconsiderable quantities in the bark of trees, dead leaves, cell walls, etc.

Table VII. includes some of the ordinary articles of vegetable dietaries, and demonstrates the need for recognition of their purin-holding capacity.

Table VII.

	Quantity used for Estimations.	Purin Nitrogen. %	Calculated as Purin bodies. %	Grams per kilo.	Grains per lb.
Cereals :					
Bread—white ...	500 grams	no trace	—	—	—
Oatmeal	250 ,,	0·0212	0·0530	0·530	3·4563
		0·0210			
Rice	500 ,,	no trace	—	—	—
Pulses :					
Peameal	250 ,,	0·0156	0·0390	0·390	2·5413
		0·0278			
		0·0247			
Beans (Haricot).	500 ,,	0·0250	0·0637	0·6375	4·1661
		0·0252			
		0·0250			
Lentils	500 ,,	0·0250	0·0637	0·6375	4·1661
		0·0252			
Lentils (malted).	500 ,,	0·0150	0·0375	0·3755	2·3340
		0·0156			
Roots and Tubers :					
Potatoes	1 kilo	0·0008	0·0020	0·0200	0·1400
		0·0006			
Onions	250 grams	0·0031	0·0090	0·090	0·0630
		0·0040			
Tapioca	250 ,,	no trace	—	—	—
Green Vegetables :					
Cabbage	large head	,,	—	—	—
Lettuce	,,	,,	—	—	—
Cauliflower	,,	,,	—	—	—
Asparagus (cooked)	700 grams	0·0086	0·0215	0·2150	1·5050

The Purins Contained in Beverages.

So many analyses of tea, coffee, and cocoa exist, that it is unnecessary to add to them. From the following table it will be seen that although purin bodies were not found to be present in wines, they existed in fairly large amounts in other fermented liquors. Probably their presence is due to peculiarities in the yeasting process. The work of Victor Lehman is interesting in this regard. He took 300 gms. of yeast and allowed it to stand for 24 hours at ordinary-room temperature. The filtered fluid yielded traces of hypoxanthin and 0·0179 gm. guanin. Further estimations of the yeast itself at body temperature demonstrated its tendency to the formation of hypoxanthins and guanins.

The significance of these facts will be discussed under another heading. It suffices now to remark that a large percentage of the existent records of uric acid excretion are from patients or workers whose dietary included beer and porter. Table VIII. contains the amount of purins found in the beverages examined.

Table VIII.

	Quantity used for estimation.	Purin N %	Purin %	Purin grams per litre.	Purin grains per pint.
Beers:					
Lager Beer	1 litre	0·0050	0·0125	0·1250	1·0955
	,,	0·0053			
Lager drink	,,	0·0020	0·0050	0·0500	0·1533
	,,	0·0021			
Pale Ale ...	,,	0·0059	0·0145	0·1450	1·2708
	,,	0·0056			
Porter ...	,,	0·0060	0·0155	0·1550	1·3578
	,,	0·0062			
Wines:					
Claret ...	½ litre	no trace	—	—	—
Volnay ...	,,	,,	—	—	—
Sherry ...	,,	,,	—	—	—
Port... ... (Commendador)	,,	,,	—	—	—
Tea, &c.:		Methyl-purins.		Per tea-cup.	
Ceylon ...	—	0·0164	0·0587	0·0805	1·210
Indian ...	—	0·0147	0·0500	0·0700	1·050
China ...	—	0·0107	0·0365	0·0460	0·750
Coffee ...	—	0·0294	0·1000	0·1100	1·700

Milk, butter, eggs, and cheese should be placed under the heading of animal foods, but they are best estimated by the method which was employed for vegetables, and so may be considered as a separate class. *Eggs* contain no free purin or purin-yielding substance. *Butter* and *cheese* are derivatives from milk, and so may hold traces of nuclein bodies. The percentages of casein and water vary in different samples of butter, and the same remark

may be applied to cheese. When the curd is pre sent, any xanthin present in the milk will pass into the slightly acid whey, just as the process of churning or separation assists their removal from the butter, and hence only minute traces can remain in these two substances. In milk, Petren was unable to find any purin bodies, but Burian and Schur by *complete* removal of all the albuminous bodies found 0·0014gm. purin-N. per litre. An estimation of my own yielded 0·0020gm. N. per litre. The quantities are very small, but are probably correct, as Barthel, of Stockholm (private communication), by use of the centrifuge has enumerated at least 100,000,000 leucocytes per kilo of ordinary cow's milk.

These four articles of diet form together our most valuable m eans of withholding purin substances from the body, and yet allow the provision of a diet at once digestible, easily absorbed and capable of maintaining nitrogenous equilibrium.

CHAPTER IV.

The Action of Food Purins.

When Liebeg first introduced his "extract of meat," many investigations were undertaken to demonstrate the effect of the extractives upon the body, but since then they have received little attention. Current handbooks upon dietetics regard them as somewhat necessary evils, and almost negative in character. They are considered to yield no potential energy, to exert no influence upon the circulatory or nervous systems, although they may remove the feeling of fatigue and slightly aid digestion. Their excretion throws extra work upon the metabolic and eliminative organs, and hence their passage through the organism is accompanied by a loss of energy. Rarely is any reference made to their cumulative after effects, which are possibly of deep import.

Alimentary system. The food purins are powerful sapients, and both directly and indirectly increase the salivary flow. The secretion induced is, however, more watery than digestive. The gastric juice, according to Pawlow is similarly affected, but Potapow-Procaitis has recently observed that although meat extractives increase the total quantity, they invoke little secretion of pepsin, and only affect the production of hydrochloric acid. He fed a dog (which had a Pawlow's fistula) upon milk,

boiled egg albumin, and meat that had been saturated with water in order to remove all soluble substances. Though Pawlow had determined that the amount of gastric secretion was proportional to the digestive intensity required by the ingesta, in Potapow's experiment little peptic secretion was induced. When, however, meat-extract or dextrin was added to the other food, a copious flow of pepsin immediately followed. Mark Schnorf, however, finds that *pure* dextrin does not cause peptic secretion, and ascribes the action of the ordinary dextrin to certain accompanying *extractive* bodies. Lehman had severe diarrhœic attacks after taking large quantities of Liebig's extract, but these were probably due to the mineral constituents. Pure adenin causes, in the dog, intense inflammation of the mucous membranes of the stomach and intestines. Uric acid produces irritation of the gastric and intestinal mucosa and in some cases slight diarrhœa.

Hypoxanthin and xanthin are readily dissolved by the acid secretions of the stomach and thus are quickly absorbed. Xanthin, however, is less soluble than hypoxanthin. Some as yet unpublished experiments appear to indicate that in man guanin is not easily absorbed. The nucleoproteids may be absorbed unaltered by the intestinal mucous membrane or be split up by the pancreatic juices and their phosphorus excreted in the fæces.

Mochizucki has shown that rectal enemata containing emulsions of thymus gland lead to increased uric acid excretion in man. Mendel, Underhill, and

White have obtained allantoin excretion in animals after a similar procedure, but they did not obtain any increased uric acid output after the rectal injection of nucleic acid. Kuelnau injected thymus emulsions and thymus nuclein into the peritoneal cavity of a dog and found an increased output of uric acid. Mendel, Underhill and White have recently observed an excess of allantoin after the intra-peritoneal injection of nucleic acid in dogs. After the injections there was vomiting and a slight rise in temperature.

Caffeine and theobromine do not exert any direct action upon the digestive functions, but after large doses, through overstimulation of the intestinal muscles, cramp and vasomotor disturbances may occur, followed by loss of the intestinal secretions.

Circulatory system. On the vascular system, the methyl-purins exert the more pronounced action. The earlier workers in this field concluded that these bodies directly stimulate the heart, augment its force, lessen the pulse-rate, and increase the blood-pressure. Parisot ascribed the whole action to a vasotonic origin, and Vinci obtained, after injections into normal animals, a constant rise of peripheral vascular pressure. Cushny and Van Naten now consider, however, that the most characteristic features of the action of caffeine are the acceleration of the rhythm, and a decreased strength of contraction in the auricle and later in the ventricle, accompanied or followed by a lessened degree of dilatation in diastole. In their experiments, after 0·2–1gm. a dog's heart became irregular, and the auricles and

ventricles beat arhythmically. Larger doses increased the irregularity and caused fibrillary contractions. The acceleration results from the stimulation of the so-called excitomotor apparatus of the heart. The lessened contraction may be due in part to the acceleration, and thus be considered as a secondary effect of the increased irritability of the vasomotor area, but it may be also caused by the *direct* action of caffeine upon the muscle of the auricles and ventricles. The whole action of caffeine upon the mammalian heart thus appears to consist in a descending stimulation which begins in the excitomotor area at the junction of the auricles and great veins, extends into the auricles and finally into the ventricles. The effects can be therefore explained by *direct* action on the cardiac muscle itself, without the necessity of appealing to any nervous apparatus.

Santesson, working with a cannula in the pericardium, finds that administration of caffeine causes increase of pressure and of cardiac rhythm. From the comparative effect of digitalin and cardiac poisons he concludes that as caffeine only increases the systolic part of the contractions, it exerts a direct and tonic action on the cardiac muscle. Mitchell Bruce regards tea, coffee and cocoa as extrinsic cardiac poisons.

As to the purins proper, Gautier states that *xanthin* excites cardiac muscle, but Baldi found it absolutely inactive on the frog's heart, although allantoin appeared to be slightly excitatory. Adenin, however, when subcutaneously injected,

strengthens the beat of the dog's heart. Hedbom observed an increased tonus and rhythm of the isolated heart, following the application of a watery splenic extract. Lehman, after drinking varying amounts of beef extract, found no alteration in the rapidity or force of the pulse. Bunge obtained similar results, but Kemmerich observed a slight rise in pulse frequency. Hutchison ascribes any cardiac effects to the hot water in which the meat extracts are usually taken, or to the effects of constant sipping.

Blood. Milroy and Malcolm observed in young rabbits, killed 2—10 days after the injection of 0·2—0·5gm. of nucleic acid, an accumulation of white cells in the pulmonary capillaries, an increase in the finely granular myelocytes in the marrow and a diminution in the coarsely granular oxyphile cells. The number of granules in the cells was also lessened and the usual oxyphile granules appeared to more readily take the basophile stains. Adenin, guanin and cytosin obtained from thymus nuclein produced similar results. In guinea pigs, however, the effects were neither so constant nor noticeable. L. Hue, in 1898 (proceedings of the Physiological Society), observed that when the Drosera was fed upon nucleic acid although the basophile chromatin segments were unaltered, an extremely copious secretion appeared, accompanied by rapid bending of the tentacles. Except very slight protoplasmic vacuolation, nuclein itself, however, produced no cytological changes.

Bang, after intravenous injection of guanylic acid

(from pancreas) into dogs, observed immediate excitation followed by temporary narcosis. The blood pressure fell quickly and the pulse became smaller. After varying periods the heart beat gradually became more forcible and the blood pressure normal. The rate of coagulation was considerably lengthened. The urine was distinctly alkaline and contained albumin. Mendel, Underhill and White obtained similar results after nucleic acid prepared from the wheat embryo. They also observed an increase in the flow of lymph and in the percentage of its total solids after intravenous injections of vegetable nucleic acid.

The recent work upon the caffeine group is interesting in relation to the action of the xanthin bodies. If the former exert a tonic action upon the cardiac muscle cells, it is not unlikely that when they become demethylated in the body, they may equally as the other purins act as slight irritants to the tissues. Additionally, the purin bodies are constantly taken with the food, they throw considerable work upon the metabolic and excretory functions and if at any time these are sub-normal, the unexcreted purins may exert cumulative effects upon the circulatory organs and the blood.

Respiratory System. In 1859, Edward Smith and Hoppe-Seyler found that tea and coffee excited the respiratory functions, and caused an increased output of CO_2, the effect lasting for over an hour. In both cases, however, the experiments were made with apparatus now considered unreliable. Smith wore a mask which covered the face, and which was con-

nected by rubber tubes with the absorptive media. The method was trying and tiring, and as Hoppe-Seyler has since pointed out, gave abnormal results. No evidence exists as to the action of the other purins either upon the CO_2 output or upon the respiratory mechanism. Levén in 1868, Giraud in 1881, and Parisot in 1890 observed that the administration of caffeine to animals caused acceleration of the respiratory movements, but after toxic doses the respiration ceased before the heart was arrested. In tea and coffee, however, there are other bodies which cause increased respiratory exchanges, for Binz and Archangelsky find that a distillate of tea and coffee, free from caffeine and thein, augments both the output of CO_2 and the number of respirations. Heerlein had earlier pointed out that the coffee distillate also affected the nervous system, but Lehman and Wilhelm, in 1898, considered that the distillates were inert. Still, as all aromatic substances are tissue irritants, and increase reflex excitability, it is probable that the contents of the distillate are responsible for the observed effects. The increase in the elimination of CO_2 found in the earlier experiments may thus, in some degree, be due to constituents of tea and coffee other than caffeine and theobromine, as well as to the methods of experiment and analysis which present the objections previously noted.

Genito-urinary system. That the methyl-xanthins are useful diuretics is a fact long since appreciated. The recent works of Anthen and Gottlieb and Magnus show that the methyl-purins act as direct

excitants of the renal parenchyma, and lead to an increased excretion of the nitrogenous elements, especially of urea and uric acid. Ach finds that in rabbits they are less effective. Uric acid appears to be without effect on the renal cells, although it is quite possible it may possess some lymphagogic properties.

After the injection into rabbits of small doses of hypoxanthin, Gaucher observed inflammatory changes in the kidney parenchyma, and these were confirmed later by Kolisch. Croftan produced under similar conditions interstitial as well as parenchymatous nephritis, albuminuria, nephritic endarteritis, and small-celled infiltration of the intima and adventitia. These changes were accompanied by an increased blood pressure and cardiac hypertrophy. In some of the animals distinct emaciation occurred. Parallel investigations with uric acid gave negative results. Upon these observations is reared the imposing theory of the alloxur-base causation of the chronic forms of nephritis, which now, however, is somewhat discredited, since it has been impossible to prove the existence of large quantities of the purin bodies in the human blood stream.

The aminopurins, when obtained from thymus or pancreas are excreted in unaltered condition, but when administered in a pure form, adenin causes in the dog an intense inflammation of the tubular cells, and uratic deposits in the lumen and interstices of the renal epithelium. Interesting in this relation is the work of Steudel, who considers that

thymin through its close connection with the hypothetical "uracil" may be akin to pyrimidin derivatives. In the hope of obtaining purin bodies by systemic synthesis, he fed a dog with methyluracil, nitro-uracil, dioxypyrimidin, etc., but the urinary purins were not increased, and no toxic symptoms were observed. When, however, he added an amido-group, as di- or tri- amino-oxy-pyrimidin, 0·1–0·2gms. were lethal to rats, and in the kidney tubules there was deposited an almost insoluble salt of the unchanged base.

Nervous system. In small doses caffeine and theobromine are cerebral excitants, and after toxic quantities the medullary centres are directly affected, the motor functions of the cord accelerated and the peripheral nerves, particularly the pneumogastric, paralysed. Baldi supposes that their action is due to the liberation of a methyl group. This group has no precise stimulative effect, the excitation being dependent upon the way in which protoplasmic activity causes its liberation, and the particular portion of the purin nucleus to which it is attached. Xanthin and hypoxanthin do not increase the excitability of the spinal cord, but Gautier obtained excitation of reflexes and tetanus after the injection of hypoxanthin in *guinea-pigs*. According to Salomon and Krüger the alloxur bodies cause migraine, and after large doses of uric acid, taken for experimental purposes, I have myself had headache lasting for several hours.

Muscular system. The purin nucleus itself exerts a specific action upon muscular tissue, and its effect

is quite independent of any attached groups. Kobert and Rossbach observed that the application of hypoxanthin to frog's muscles after severe muscular efforts, caused renewed activity, and inferred that it removed the sense of fatigue and stimulated muscular tissue. Filehne noticed muscle rigidity and tetanus in frogs after hypoxanthin, and rigidity followed by cardiac arrest after xanthin. Guanin and uric acid up to 100mgm. were inert.

Mitscherlich et Bennett and Filehne, in 1887, observed similar results with di-oxy-purins; and Paschkis states that the toxicity becomes more intense from the hypo- to the methyl-xanthins. Johannsen, after caffeine injection in frogs, obtained muscular rigidity and loss of excitability, and by analogy between blood and muscle plasma coagulation, Klempner thought that caffeine thus evoked the development of a coagulative ferment. Although Rossbach and Hartenack were unable to confirm these results, they have since been substantiated. Leblond observed that in frogs, caffeine produced a period of transitory rigidity followed by tonic and tetanic convulsions and diminished excitability, and thought that the action was first upon nervous and later upon muscular tissue. Lusini states that the minimum toxic and fatal doses decrease from the mono- to the tri-methyl xanthins, and that the resistance of the muscle against fatigue increases in an inverse direction, and Fére concludes that although muscular power is for a time increased, yet caffeine causes an earlier appearance of fatigue than that which normally occurs. Baldi points out

that while the methyl group is responsible for the excitation, it is the xanthin or purin nucleus alone which produces the rigidity and hyper-excitability. Finally, Schmiedeberg has published the results of an interesting investigation upon the rôle played by the oxy and alkyl attachment to the purin nucleus, and shows that whilst these groups may alter the intensity of action, the purin nucleus still exerts its own specific action upon muscular tissue.

In experiments with substances akin to the purin bodies two conditions must, however, be always remembered; (1) that the relative actions of the several purins may be due to their varying solubilities, which in the one case allows free circulation in the lymph spaces, and in another permits only partial absorption by the individual cells; and (2) that they may form weak salts with some of the lymph constituents, and so obtain augmented or diminished powers.

Metabolism. The purin bodies do not appear to exert any direct influence on either carbohydrate or nitrogenous metabolism. Acting through the nervour system, caffeine is said to raise the tonus of the tissues, and so permit the organism to utilise its reserves. Indirectly, however, they invoke an output of metabolic energy to ensure their early removal from the body. They are not used for the production of cell nuclein.

In regard to the "forced feeding" treatment of tuberculosis and the small percentage of infective disorders amongst gouty patients, it would be of value to know if an excess of purin bodies in the

bloodstream increases or diminishes the *immunity* of the individual, or if there is any alteration in bacterial cultures, when purins form one of the constituents of the media. Bendix has lately shown that the presence of uric acid neither retards nor diminishes the growth of certain micro-organisms, and I have been able to confirm these results, especially as regards several varieties of micrococci. When uric acid, hypoxanthin and guanin were severally added to the nutrient media, the resultant growth in no way differed from that of the control tubes.

CHAPTER V.

The Comparative Effect of Purin Bodies upon the Production of CO_2.

As previously cited, earlier investigators observed an increased production of CO_2 after the ingestion of tea and coffee. These increases were obtained, however, by the use of unsuitable apparatus and by unsatisfactory methods of analysis. It would thus be of interest to compare the results of modern experimental methods with those already recorded, and preferably to employ pure caffeine instead of an infusion of coffee. With the object of making such comparison, and also to ascertain the action of the other purins upon the carbohydrate metabolism, the following studies were devised.

The subject of experiment rose about 7-30 a.m., walked an English mile, and still fasting, entered the Sondén-Tigerstedt respiration chamber about 8-30 a.m., and lay absolutely still upon a mattress for 30 minutes, covered only with a light felt rug. One sample of air was taken five minutes after entry, and another thirty-five minutes later. The temperature and pulse-rate were observed at the commencement and at the close of each experiment. The analyses were made by a Sondén-Peterson's apparatus.

W. H., of Manchester.

Nov. 18, 1901, 8–0 a.m., ½gm. Benz. Natr. Caffeinicus. CO_2 excretion.
8–30 a.m., ½ gm. ,,
8–35 a.m., Temp. 36·5° C., pulse 80.
9–5 a.m., Temp. 37·8° C., pulse 78. 14·6 gms.

Sensations of warmth in abdomen and over the whole surface of the body. Intense headache, which lasted the whole day. Muscular fibrillation in muscles of the back and thigh.

Nov. 19, 8–35 a.m., Temp. 36·5° C., pulse 80.
9–5 a.m., Temp. 36·5° C., pulse 78. 11·2 gms.

Nov. 20, 7–30 a.m., 0·25 gm. Hypoxanthin in slightly alkaline solution.
8–15 a.m., 0·25 gm. Hypoxanthin in slightly alkaline solution.
8–20 a.m., Temp., 36·5° C., pulse 76.
8–50 a.m., Temp. 36·5° C., pulse 76. 11·1 gms.

Slight fulness in head, and a feeling of stiffness over the whole body.

Nov. 26, 7–45 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8–20 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8–30 a.m., Temp. 37° C., pulse 84.
9–0 a.m., Temp. 36·5° C., pulse 84. 14·3 gms.

There was distinct headache and confused ideas. Sensation of warmth in the stomach.

Nov. 28, 7–45 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8–30 a.m., 0·25 gm. uric acid partially dissolved in alkaline solution.
8–33 a.m., Temp. 37° C., pulse 84.
9–3 a.m., Temp. 36·5° C., pulse 84. 11·6 gms.
Slight headache, sensory disturbances in abdomen.
Nov. 29, 7–45 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8–15 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8–26 a.m., Temp. 36·6° C., pulse 84.
8–56 a.m., Temp. 36·5° C., pulse 72. 14·3 gms.
Fulness in head; loss of muscular sense; confused ideas.
Nov. 30, 7–45 a.m., 0·5 gm. uric acid, entirely dissolved in sodium carbonate solution.
8–15 a.m., 0·5 gm. uric acid, entirely dissolved in sodium carbonate solution.
8–35 a.m., Temp. 36·8° C., pulse 90.
9–5 a.m., Temp. 36·5° C., pulse 76. 11·0 gms.

Dr. Friherre A. C., of Finland.

Normal rest value 9·8
10·3 gms. 10·3 gms.
CO_2 excretion.
10·0
Nov. 21, 7–30 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8–0 a.m., 0·5 gm. Benz. Natr. Caffeinicus.
8–5 a.m., Temp. 36·1° C., pulse 60.
8–35 a.m., Temp. 36·1° C., pulse 60 (fuller) 10·7 gms.
No headache; slight warmth over whole skin area; no fibrillations.

Nov. 25, 7–30 a.m., 1 grm. Benz. Natr. Caffeinicus.
8–0 a.m., 1 grm. Benz. Natr. Caffeinicus.
8–12 a.m., Temp. 36·2° C., pulse 62.
8–42 a.m., Temp. 36·2° C., pulse 62. 12·3 gms.

Headache; abdominal warmth, followed by diarrhœa in the later part of the day.

Professor SANTESSON, OF STOCKHOLM.

Dec. 5, 8–25 a.m., Temp. 36·8° C., pulse 56.
8–55 a.m., Temp. 36·8° C., pulse 56. 10·5 gms.
Dec. 7, 7–55 a.m., ½ gm. Benz. Natr. Caffeinicus.
8–25 a.m., ½ gm. Benz. Natr. Caffeinicus.
8–35 a.m., Temp. 37·0° C., pulse 56.
9–5 a.m., Temp. 37·1° C., pulse 57. 12·5 gms.

Audition slightly augmented, also sensation of body warmth. No headache.

The results show that my early morning CO_2 excretion is about 11 grammes per half-hour. The values of Professor S. and Dr. C. are slightly lower, as also their pulse-rate and temperature. With Professor S. and myself, caffeine caused an increased production of CO_2 in average doses, but an almost toxic dose was required before any effect was manifest upon Dr. C. It would appear, therefore, that although caffeine may incite increased combustion, it acts variously upon different individuals. The results recorded do not indicate whether the excitation is directly upon the metabolic organs, or indirectly through the nervous system. At all events, caffeine increases the elimination of CO_2 in many cases—a fact of interest in the consideration

of the treatments available for the alterations of cell-activities during stasis.

Hypoxanthin, the monoxypurin, yielded negative results, and uric acid, the trioxypurin, may probably be placed in the same category. The chemical constitution of these bodies and their behaviour during metabolism, pointed to the probability of their inactivity, but the observations have yielded results which indicate the limited rôle of the purin-nucleus, and allow us to ascribe the effects of caffeine upon respiration to the action of its methyl-groups. That the latter mainly excite nervous tissues is certain, but that they may also directly affect cellular processes is not disproved and not improbable. In these experiments, however, the action of uric acid was somewhat irregular. On the first occasion, when the uric acid was only partially dissolved, there was a sensation of warmth in the alimentary canal, which, apart from the intense headache, would be sufficient to account for the rise obtained. There was no headache on the second day, but the stomach sensations were present; for the third experiment the uric acid was completely dissolved in 340cc. of $Na_2\ CO_3$ solution, and neither headache nor other symptoms interfered with quiet respiration and general comfort. Possibly my body became rapidly tolerant of the repeated doses of uric acid.

That the substances taken were duly absorbed appears from the following records:—

TABLE X.

Nov. 21, 7-30 a.m. Urine passed:
„ 21, 8-15 a.m. 0·5 gm. Hypoxanthin (0·20 purin N.)

	Urine.	Total N.	Total purin Nitrogen.
10 a.m.—12 noon	415 cc. ...	3·949 ...	0·1227
12 noon—3 p.m.	100 cc. ...	2·548 ...	0·0792
3 p.m.—7-30 a.m., Nov. 22	650 cc. ...	12·989 ...	0·1560
	1165 cc.	19·486	0·3579

TABLE XI.

Nov. 27, 7-30—8-0 a.m., 0·5 gm. uric acid (0·166 purin N.)

	Urine.	Total purin Nitrogen.
	10 a.m., 250 cc.	0·0330
	2 p.m., 150 cc.	0·0396
	8 p.m., 230 cc.	0·0606
	10-30 p.m., 150 cc.	0·0376
Nov. 28,	7-30 a.m., 270 cc.	0·0712
	1050 cc.	0·2420

It will be seen from later experiments, Table XIV., that my endogenous purin-N. is 0·1625 per 24 hours. During these experiments I ate purin free food, with the exception of 300gms. of veal on the hypoxanthin day. This latter yields about one-half its purin nitrogen as urinary purin-N. (Table IV.), therefore, 0·075 of the urinary purin would be from this source, 0·1625 + 0·075 = 0·2375. The total quantity of urinary purin after the hypoxanthin was 0·3579. This, minus 0·2375 = 0·1224 N., directly due to the hypoxanthin ingested. As the N. of 0·5gm. hypoxanthin = 0·200, it will be seen that 60 per cent. of the hypoxanthin N. was voided in the

urine during the 24 hours. This confirms similar results by Burian and Schur. Equally, when the uric acid was taken, the purin-N. excretion was 0·2420. On this day, only eggs, milk, bread, cheese and butter were eaten, and the endogenous purin-N. = 0·1625. The difference of 0·0795 came, therefore, from the uric acid: 0·5gm. of uric acid = 0·1666 N., and hence during the succeeding 24 hours 47·7 per cent. of the uric acid ingested was excreted as urinary purin. It will be shown later, that the remainder was probably excreted as urea.

Sœtbeer and Ibrahim have recently published experiments showing that uric acid is not absorbed when taken per the mouth. The results obtained are at distinct variance with those of other workers as well as those above stated, and confirmation of Sœtbeer's figures are necessary before we can accept them. If the uric acid was not absorbed, it should have been recoverable from the fæces, but this Sœtbeer and Ibrahim did not attempt. Neither the total purin nitrogen nor the xanthin bases are given, so that conclusive deduction from their figures is impossible.

A noticeable feature of these absorptive results, is the rapidity of their excretion. Almost the whole of the expected amount of hypoxanthin was eliminated as urinary purin within a few hours. Later experiments have given somewhat similar results, but the subject needs wider application. The administration of these substances might be of use as an indicator of individual purin metabolism. A known quantity given with purin-free food,

should, under ordinary circumstances, be excreted as urinary purin to the extent of 50 per cent. in x hours. The integrity of the liver and kidney cell activities would be a matter of easy induction from the results obtained, and some clue would be gained as to the tonicity of the vascular circulation in these organs. The application of this suggestion will appear more feasible when purin metabolism has been discussed.

CHAPTER VI.

The Effect of Continued Daily Injections of Purin-Bodies in Rabbits.

These experiments were undertaken with the object of recognising any changes in the liver, marrow and nerve cells after the injection of hypoxanthin, etc., and of repeating previous investigations upon the kidney and blood-pressure.

Dosage. The quantity injected corresponded with the average daily amount (per kilo) taken by an adult man in his food. This would be a large dose for an animal accustomed to small quantities of purins in its food, but yet not approach the toxic doses of Milroy and Malcolm. It is difficult to conclude how much Croftan administered, as the weights of the animals are not stated. The results of toxic injections are certainly of value and interest, but as the purin action under general conditions is perhaps more cumulative than momentary, and through overstrain of cellular functions may produce its effect indirectly, an attempt to obtain some less pronounced tissue changes should lead to a better knowledge of cell reactions in pathological metabolism.

The injections were made and the operations

performed at Carolinska Institutet, Stockholm. Four young rabbits were procured six days before the experiment commenced, kept in separate cages, fed on the same daily quantities of oats throughout, and their urine examined regularly.

Each day from September 23rd to November 12th inclusive,

Rabbit 1 (1480gms.) received 5cc. of 0·025 slightly alkaline solution of hypoxanthin (for a man weighing 68 kilos = 1·133gms.).
„ 2 (1546gms.) received 1cc. of 0·025 slightly alkaline solution of hypoxanthin (for a man weighing 68 kilos = 0·2193gms.).
„ 3 (1713gms.) 1cc. of 0·025 of guanin solution (for a man weighing 68 kilos = 0·2193gms.).
„ 4 (2025gms.) was employed as a control.

The hypoxanthin used for No. 1 was made by the usual process from fresh beef, that for No. 2 was obtained from E. Merck., of Darmstadt. The guanin was prepared from the scales of *alburnus lucidus*, which, after boiling in 5 per cent. NaOH for four hours, precipitation with freshly-prepared cuprous oxide, and decomposition by H_2S, yielded guanin upon the addition of strong ammonia to the H_2S freed filtrate. It contained 42·46 per cent. nitrogen (Kjeldahl) and was free from all albuminous material.

The rabbits were weighed each day at 3 p.m. Although they all received the same amount of food, while the control animal had gained 145 grams at the end of the experiment, No. 1 and No. 3 had only increased 60 and 52 grams respectively, and

No. 2 had lost 206 grams. The effects of the injections were thus reflected in their weights. The control rabbit increased gradually in weight, No. 1 gained up to a certain level and then just managed to maintain it, but No. 2 during the last 20 days rapidly emaciated. The animal in which guanin was injected was subject to marked diurnal or di-weekly increases or diminutions of weight.

On the 43rd day blood films were made from each rabbit, and on the following morning, 4—6 hours after a meal, blood pressure tracings were taken. After death, the heart, vessels, kidneys, lungs, marrow, and brain were removed for histological examination, and the muscles boiled with 0·5 per cent. H_2SO_4 to ascertain their purin contents. These did not differ from the average normal amount.

On November 13th, Professor Johannsen very kindly assisted me in the necessary operations for the record of blood pressures. To avoid any circulatory depression, only local anæsthetics for the skin incision were used.

TABLE XII.

BLOOD PRESSURE RESULTS, NOVEMBER, 13TH, 1901.

Rabbit I. 1540gms.	Rabbit II. 1340gms.	Rabbit III. 1765gms
1-25p.m., 104m.m.	2-10p.m., 100m.m.	2-50p.m., 104m.m.
1-30p.m., 106m.m.	2-15p.m., 104m.m.	2-51p.m., 98m.m.
1-40p.m., 110m.m.	2-20p.m., 102m.m.	2-52p.m., 96m.m.
1-45p.m., 108m.m.	2-27p.m., 106m.m.	2-55p.m., 96m.m.
	2-30p.m., 98m.m.	

Rabbit IV. 2110gms.

12-10p.m., 122m.m.
12-15p.m., 116m.m.
12-40p.m., 118m.m.
12-43p.m., 118m.m. 10cc. hypoxanthin (0·5%)
12-44p.m., 122m.m. solution injected.
12-45p.m., 122m.m. (intravenous).
12-50p.m., 118m.m.
12-51p.m., 118m.m.
12-52p.m., 118m.m.

These figures show that the blood-pressure is not altered by purin bodies, either immediately or remotely. Croftan, however, states that cardiac hypertrophy and increased blood-pressure are caused by the alloxuric bases. In his rabbits he found blood pressures of 104, 107, and 108m.m. My results are practically the same, but Croftan takes the normal B.P. at 60—90m.m., and so shows an increase of 20—40m.m. The pressure in my control animal was 120m.m., but unfortunately Croftan's figures cannot be compared, as he does not record the weights of his rabbits. I have examined the reports of some 500 experiments conducted upon English, German and Scandinavian rabbits, and their average B.P. is 100—135m.m. Croftan's figures thus appear to point an entirely opposite conclusion to the one he draws.

Post-mortem Appearances.

Rabbit I.

Heart: pale and flabby; no apparent increase in size.

Lungs: normal.

Liver: hæmostasis.
Kidneys: cortex very pale, little distinction between the two zones.
Marrow: pale and anæmic.

RABBIT II.

Heart: pale and flabby.
Lungs: normal.
Liver: hæmostasis.
Kidneys: same as No. 1.
Marrow: pale.

RABBIT III.

All the organs appeared normal, except for slight passive congestion at the base of the right lung.

Blood-films. The blood was obtained from the median artery of the ear, the films fixed with formalin vapour, stained for two minutes with 1 per cent. saturated watery solution of methylene blue and differentiated with a 1 per cent. solution of erythrosin and absolute alcohol. All the histological appearances are described in comparison with those of the control animal. In rabbits 1 and 2, the lymphocytes and polynuclear leucocytes were markedly increased, and cells which appeared of a basophile character were very numerous. There were no changes in the red blood corpuscles.

Histology. The following organs were examined: liver, lungs, heart, kidneys, marrow, blood vessels, and brain. They were fixed (1) in absolute alcohol, chloroform, and acetic acid; (2) in mercuric chloride and glacial acetic acid; and (3) in mercuric chloride

and picric acid. Afterwards they were passed through 30, 40, 50, 60, 70, 82 and 95 per cent. absolute alcohol and carbon bisulphide, carbon bisulphide, carbon bisulphide and paraffin, and finally embedded in 58°C paraffin. Sections of 2—5 (u) were cut, and coloured with the following stains:—

(1) Hæmatoxylin and eosin.
(2) Hæmatoxylin and eosin and orange G.
(3) Methylene blue (1 per cent.) and eosin ($\frac{1}{2}$ per cent. in alcohol).
(4) Iron alum hæmatoxylin, acid fuchsin and orange G.
(5) Toluidin blue (2 per cent.) and differentiation in $\frac{1}{2}$ per cent. erythrosin.
(6) Weigert's fuchsin—resorcin solution.

In rabbit 1 and 2, the heart, large blood vessels and nerve cells did not show any distinct changes. In rabbit 2, there was a slight bronchitis, and the brain was hyperæmic.

The *kidneys* of both animals showed degenerative changes in the cells of the convoluted tubules, marked hyperæmia, a commencing proliferation of the intima of the *smallest* arterioles, but no interstitial nephritis, and no glomerular changes.

The *marrow* was crowded with lymphocytes and nucleated red-blood corpuscles. The cell forms intermediate between the lymphocyte and myelocyte were numerous, and showed a tendency to take basic dyes. Most noteworthy was the unusually large number of "Riesen-zellen," some of which showed signs of degeneration. Stained by Weigert's resorcin

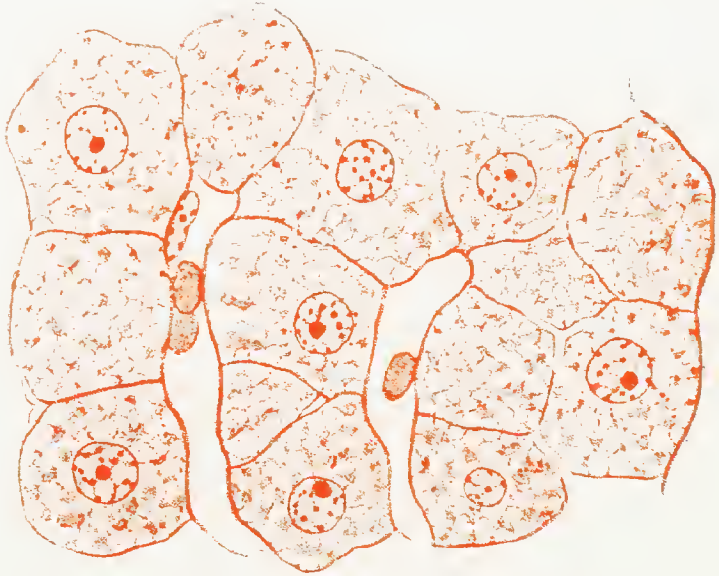
method, the intima of the smaller blood vessels appeared to be thickened.

Liver. Hyperæmia was well marked in both livers, but there was no signs of any interstitial changes. Stained with eosin and methylene blue, many scattered foci could be seen where the eosin had stained more deeply than normal, the result, probably, of slight degeneration.

In other places the capillaries were very dilated, the cells had almost lost their rowed arrangement, and were separated on all sides by the engorged vessels. Stained with iron-alum, hæmatoxylin, acid fuchsin orange method, the cells in these areas showed an altered arrangement of their protoplasm, which was gathered chiefly around the nucleus. The remaining cell contents appeared to be clumped together as if upshaken by a strong electric current, and showed degenerative changes. There was considerable vacuolation. The nuclei were swollen and distorted and contained sharply-staining nucleoli, principally polar, and a large number of cells had two nuclei.

It was possible also to find odd cells which were almost necrosed, and around which leucocytes had collected. Methylene blue showed some extrusion of nuclear bodies. The portal canal and bile ducts were normal.

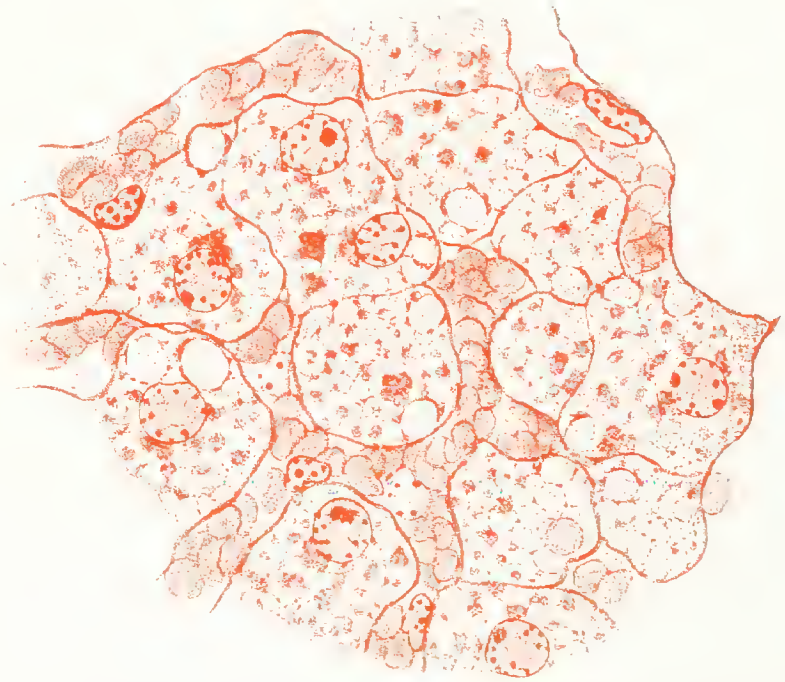
The pathological changes in the cells of the blood, lymphoid and myeloid tissues do not yet permit of full explanation, as Dominici's recent experiments and criticism of Ehrlich's theories tend to show. But one may make the general inference that in



Liver (Rabbit) Normal

Drawn by Ericken Johansson.
Artist to Histologiska Afdelningen
Carolinska Institutet, Stockholm.

Zeiss 1/12th Immersion
Oc 3. Camera lucida.
Iron alum hematoxylin acid
fuchsin - orange



Liver (rabbit, after hypoxanthin injection) shewing hyperæmia, degenerative changes in the protoplasm, vacuoles, swollen and distorted nuclei, regeneration

these rabbits a distinct cellular reaction was directed against bodies of a toxic nature. The alterations in the liver parenchyma suggest the presence of some exogenous or endogenous cell poison, but certain morbid manifestations may follow overworked or overstrained organs, and the slowness of growth in rabbits 1 and 2 is somewhat significant.

Whether it is reasonable to assume that the purins exert a similar influence in man is hard to say. The exogenous purin in the rabbit is, as a general rule, very much less than in man; and it could be well argued that human cellular tissues are more immune to these bodies than those of the rabbit. Mitchell Bruce, Bier, Jelks, and many others think that the kidney, cardiac and vascular changes in nephritis are due to an irritant of a chemical nature. Croftan and Kolisch consider that alloxuric bases are the specific irritant, but there is no evidence obtained by reliable methods which substantiates any increased circulation of these bodies in the blood or lymph stream of nephritic patients. At the same time it must be admitted that the purin bodies are not wholly harmless, and any deficiency of oxidation might readily delay the transformation of these insoluble substances into the soluble urate, and so retard their passage into the circulation.

The tissues of rabbit No. 3, into which guanin was injected, presented no noteworthy alterations.

Kochmann has recently fed several dogs on oxen-flesh alone for six to ten weeks. In one there was distinct loss in weight and in all the liver showed cloudy swelling and fatty infiltration. Broncho-

pneumonia, acute hæmorrhagic nephritis, cloudy swelling, fatty degeneration and parenchymatous inflammation of the kidney were also observed. The urine contained blood and albumin. Although he raises the question of a possible acid intoxication, his conclusions indicate that excessive flesh food as well as alcohol and lead may cause deficient liver metabolism, kidney degeneration and the consequent alterations in the excretion of uric acid.

At present there is a tendency to disregard the action of the oxypurins upon the tissues, but it must be remembered that when they are in excess they probably form unusual combinations and that such products may act as irritants. When Kochmann added carbohydrate food to the meat diet, his dogs presented few post-mortem pathological changes. Up to the present date, I have made a large number of personal experiments, and when I have taken large doses of purin bodies—such as $\frac{1}{2}$gm. of hypoxanthin, 1–5gm. of guanin, 0·5–1gm. uric acid—apparently associated symptoms of general malaise and irritability have frequently appeared.

CHAPTER VII.

The Fate of Food Purins in the Body.

The relation between the ingested food and the simplified nitrogenous bodies of the urine and fæces has been the cause of many experiments and much theory. Practical demonstration of the intermediary stages has been very slow, and it is only within recent years that chemical physiologists have demonstrated the differences between the decomposition products of proteid and nuclein. The view, that during metabolism one portion of the absorbed proteid is first anabolised into living protoplasm and the other undergoes direct katobolism, has been further developed by the belief that as all metabolic processes are intracellular, no katabolism occurs except as a result of cell influence. Whether, however, as some cytologists affirm, the proteid first undergoes entire anabolism, or as others consider, is in part *directly* katabolised, in both cases, as a result of cellular control, through oxidations, dehydrations, decompositions and perhaps also by synthesis, it is excreted as urea.

Wöhler and Frerichs, in 1848, from the results obtained by the injection of urates into rabbits, considered uric acid to be an intermediate body between proteid and urea. Amongst many others, Stad-

thagen, in 1887, thought that uric acid resulted from the cleavage of albumin, and that its quantity was dependent upon the amount of albumin in the food, and Mares proposed a theory of its formation by glandular cells, and its consequent expression of the secretory activity of the body. As late as 1898, the following statement appeared in a well-known book: "The production of uric acid depends upon the ingestion of proteid matter, and it makes no difference whether the proteid matter be of animal or vegetable origin. The only reason that a vegetable diet is less productive of uric acid than an animal diet, is in the fact that the former is poorer in proteid material. With the same intake of nitrogen in the two diets, there is practically no difference in the uric acid output."

On similar grounds is based Latham's theory that uric acid results from the synthesis of urea and glycocin through the stages of hydantoin and biüret. In 1899, however, Taylor showed that the amount of ingested proteid has absolutely no constant relation to the quantity of uric acid excreted, and that even with a proteid-free diet of 350gms. sago, 50gms. sugar and 100gms. butter, the urine contained 0·273gms. of uric acid per 24 hours, and Hess and Schmoll have not found increased purin excretion after the consumption of even twenty-four hen's eggs. Jerome concluded from the results of a long experiment, that "there is at present no proof that uric acid can arise in man independently of a substance containing an alloxur or purin group." Maurel, however, recently pointed out that if a con-

Wiener has lately shewn that from an experimental standpoint, a small amount of uric acid, may arise synthetically, but that the quantity is almost infinitesimal. Nuclein, on the other hand, according to the work of Burian and others, is being constantly formed from the purin-free proteids.

dition of *nitrogen hunger* is induced, the amount of nitrogen in the food exerts some influence upon the uric acid output. For instance:—

Food of	3250	calories	with	1·25gm.	N.	per day = 0·21	uric acid.
,,	3800	,,		1·00	,,	,, = 0·11	,,
,,	2500	,,		0·50	,,	,, = 0·07	,,

But as early as 1867 Gorup recognised the importance of the nuclein bodies in regard to metabolism, and Kossel, in 1881, obtained xanthin bodies from pure nuclein by hydrolytic cleavage. His declaration in 1882, that nuclein might be a source of uric acid with hypoxanthin as an intermediary body led to a series of experiments in which rabbits, cats and dogs were fed with nuclein bodies, and increased amounts of urinary purins demonstrated. The work of Horbaczewski, who showed that oxidation of fresh splenic pulp by bacterial agencies, produced uric acid of the same nitrogenous value as that of the nucleins of the tissues, and that nuclein prepared from splenic substance, dissolved in weak alkali and digested with blood at 40°C, yielded uric acid, led up to the theory that the decomposition of nuclein in the tissues yielded xanthin and hypoxanthins, and that these bodies, after oxidation, were excreted as uric acid. The increased amount of uric acid, eliminated after meals rich in nucleins and present in the urines of leucocythæmic patients, supported Horbaczewski's further contention that disintegration of leucocytes was the source of uric acid.

Parallel examinations of the blood and urine have, however, subsequently shown that leucocytosis does

not regularly accompany increased uric acid excretion, and Hutchison and Macleod have recently reported a case of leucopenia in which the alloxuric urinary nitrogen was of an average normal amount. Whilst we may thus regard leucocytic destruction as one source of urinary purin, it probably does not play such an important rôle as was formerly thought.

The supposition of Haig that the urinary uric acid is in part the expression of the uric acid contained in the food, may be cited. His conclusions are elsewhere reviewed, although they are not generally accepted.

The observations of Weintraud, Mayer, Umber, Jerome, and Hopkins and Hope have proved that thymus and pancreas, bodies rich in nucleins, cause an increased urinary purin output.

As regards the *cleavage products of nuclein*, Strauss, in 1896, obtained an increased uric acid excretion after the addition of 50gms. of meat extract to his usual diet, and Jerome recorded varied increases after lamb, steak, mutton, fowl, pigeon and partridge.

The methyl-purins of the food increase the total purins of the urine, but only slightly affect the uric acid excretion. Albanese, and Krüger and Schmidt have shown that these bodies are demethylated during metabolism, and that the 1—3—7 methyl-xanthin caffeine is excreted as di- and monomethyl-xanthin, and the 3—7 di-methyl-xanthin, theobromin, as 3 methyl-xanthin.

All foods containing purin bodies thus appear to increase the excretion of uric acid and the

xanthins. What then, is the effect of the purin-free or purin-poor foods? Milk was found by Burian and Schur to result in a very low uric acid excretion, and casein, aleuronat, and sanose by Rosenfeld and Bornstein, Brandenburg, and Schreiber to produce a similar effect. In this regard the purin excretion of children fed upon milk is interesting. Camerer reports the case of an unweaned child nearly twelve months old, whose total daily urinary purin = 0·035gm. N., and Bendix obtained 0·098gm. of uric acid (0·033gm. N.) from the urine of a three month's old child fed upon cow's milk, and in a child aged seven years I have found 0·1072 – 0·1170 urinary purin N. on a milk diet, and 0·1570 – 0·1600 on a mixed diet. Camerer estimated the effects of food upon purin excretion and arrived at the following results: —

	Urinary Purin N.
A diet of milk, cream, eggs, meat and coffee	= 0·72
„ peas, cabbage, fat, bread, butter and beer	= 0·41
„ potatoes, chestnuts, fruits, bread, butter, honey, meal and milk	= 0·36
„ meat, fruit, vegetables, eggs and cheese...	= 0·66

As coffee and beer were taken, it is impossible to draw any adequate conclusions as to the exact effect of these diets. Taylor found: —

	Uric Acid	Bases
On a mixed normal diet	0·364	0·0249
„ „ „ plus sweetbread	0·871	0·0271
„ much proteid	0·456	0·0134
„ peas and beans.....	0·462	0·0195
„ nitrogen free food	0·273	0·0066

Hence we may infer that with certain foods a minimum of uric acid is excreted, and that as such foods probably contain little purin, they make but a slight addition to the uric acid which the body produces independently of the nucleins of food-stuffs. Sivén, in 1900, on a fixed diet, furnished experimental proof of such a conclusion, but to Burian and Schur is the credit due for its substantiation through their estimations of the purin-N. of certain foods. Their results are as follows:—

	Purin nitrogen		Purin N
Brown bread.....	0·010%	Thymus	0·386%
Milk	0·004%	Liver	0·123%
Potatoes.....	0·0005%	Beef	0·063%
		Veal	0·057%
		Ham	0·063%

In white bread, rice, eggs, salad and cauliflower, they found no xanthins. By use of these latter foods they determined the endogenous urinary purins in a number of cases, and obtained results of individual constancy. When the endogenous urinary purin was estimated, and the amount of the food purin also known, they were able to explain the relation of the food purin to the urinary purin, and knowing the amount of the food purin and the total urinary purin, they were able to calculate the quantity of endogenous urinary purin. They state that with beef and liver one-half, with thymus one-fourth, and with coffee one-third of their purin-N. appears in the urine as uric acid and xanthin bases.

The xanthin bases have not received much

attention as to the cause of their occurrence in the urine. They are present only in small amounts, and research has been mainly directed to the investigation of uric acid. The terms bases and acid are, however, in this connection somewhat misleading, as uric acid is a tri-oxy-purin, and xanthin and hypoxanthin are respectively di- and mono-oxypurin, hence their relation is closer than their names signify, and they should be estimated together.

Milroy has recently recorded the interesting fact that in birds there is always a small but definite daily output of purin bases, although the uric acid excretion is mainly synthetic in origin and but little influenced by feeding with nucleic acid.

The Metabolism of Meat Purins.

The publication, in 1900, of Burian and Schur's interesting researches, led to my investigation of the metabolic changes occurring after the ingestion of those substances, other than thymus, liver, veal, beef and ham, which my earlier estimations had shown to contain considerable amounts of purin bodies. At first sight, it must be supposed that no differences would be found, but as the flesh of certain animals (rabbits, etc.) contains methyl-xanthins, actual results appeared preferable to inference.

Subject of experiment: W.H., weight, 70 kilos (11st. 5lbs.). Urine free from albumin and sugar. The 24 hours' collection was taken from and to 8 a.m., and the urines were preserved for repeated estimations by the addition of a few drops of $CHCl_3$. During the experiment ten hours' laboratory work was performed, and a walk of four English miles taken each day.

To obtain the average amount of endogenous purin excretion, a fixed purin-free diet, consisting of eggs, bread, milk, cheese, butter, rice and sugar was selected. Neither tea, coffee nor beer was taken. Table XIII. gives the results, and indicates the variations in the calorific and nitrogenous values.

TABLE XIII.

Days	Diet	Calories Approximate	Food Nitrogen Approximate	Urinary Purin N
1.	10 eggs, 360gm. bread, 1000cc. milk, 80gm. cheese, 60gm. butter	2631	22·38	0·1611
2.	10 eggs, 300gm. bread, 1000cc. milk, 80gm. cheese, 60gm. butter	2490	21·60	0·1608
3.	10 eggs, 300gm. bread, 1000cc. milk, 80gm. cheese, 60gm. butter	2490	21·60	0·1560
4.	8 eggs, 360gm. bread, 1000cc. milk, 80gm. cheese, 60gm. butter, 100gm. rice, 50gm. sugar-	3053	20·50	0·1636
5.	8 eggs, 360gm. bread, 1000cc. milk, 80gm. cheese, 60gm. butter, 100gm. rice, 50gm. sugar	3053	20·50	0·1660
6.	8 eggs, 360gm. bread, 1000cc. milk, 40gm. cheese, 60gm. butter, 50gm. sugar, 50gm. rice	2898	17·80	0·1563
7.	8 eggs, 360gm. bread, 1000cc. milk, 40gm. cheese, 60gm. butter, 50gm. sugar, 50gm. rice	2898	17·80	0·1654
8.	8 eggs, 360gm. bread, 1000cc. milk, 50gm. sugar, 50gm. rice, 40gm. cheese, 60gm. butter	2898	17·80	0·1645

It thus appears that upon an almost purin-free diet an average of 0·1623gms. purin-N. per day was excreted, or 0·4869gms. in terms of uric acid and xanthin bases, or 0·3645gm. uric acid and 0·0950 xanthin bases approximately. Such amount is apparently uninfluenced by slight alterations in the quantities of nitrogen and the calorific values of the food.

These results are in accordance with those of Hirchfield, Sivén and Burian and Schur. In each set of experiments, however, the details differed. Sivén and Burian and Schur maintained an unchanged caloric food value, with marked nitrogen variations. Hirschfield added 57 per cent. nitrogenous material and 22 per cent. calories. In my experiment I kept the nitrogen at a constant level and added 22 per cent. of calories. In all cases the urinary purin showed a constant excretion in spite of the changed dietary. The food was, it may be noted, invariably purin-free.

In Table XIV., the same fixed purin-free diet was taken, with the addition of certain meats whose amounts are separately noted. That the effect of the increased purin ingestion might be at once evident, the principal meal was taken at 7.45 a.m., when the larger portion of the meat was eaten. A light lunch preceded the next meal at 4 p.m., when the remaining purin-rich food was consumed. Bed 10 p.m. The meats were weighed raw, then steaked with fat sufficient for the purpose, and every particle of gravy, etc., preserved.

Table XIV.

1901	Urine	Total Nitrogen	Total Purin Nitrogen	Uric Acid Nitrogen	Xanthin-bases Nitrogen	P_2O_5	Remarks
Oct. 9	1260	21·8232	0·3769	—	—	—	
,, 10	995	16·2486	0·2739	—	—	—	Usual mixed food with tea,
,, 11	1215	18·4294	0·3472	—	—	—	coffee, &c.
,, 12	1010	17·5210	0·2208	—	—	—	
,, 13	1170	20·2132	0·1611	0·1454	0·0157	2·6208	Purin-free fixed diet.
,, 14	1125	20·4975	0·1608	0·1380	0·0228	2·4712	
,, 15	1255	23·5683	0·2650	0·2190	0·0460	3·7650	450gm. chicken; *uratic deposit.*
,, 16	1110	20·4680	0·1798	0·1435	0·0353	3·3858	——
,, 17	1085	19·4215	0·1560	0·1280	0·0280	3·3743	——
,, 18	1415	23·7294	0·2489	0·1995	0·0494	3·8771	530gm. plaice; *urates.*
,, 19	1140	19·6536	0·1755	0·1644	0·0111	2·9153	——
,, 20	1010	18·2966	0·1636	0·1250	0·0386	2·6260	——
,, 21	1860	22·4680	0·2480	0·2108	0·0372	5·9520	400gm. beef.
,, 22	1450	18·2990	0·1898	0·1565	0·0333	2·4460	——
,, 23	900	16·8940	0·1660	0·1270	0·0390	2·5475	——
,, 24	1150	17·2368	0·1857	0·1391	0·0466	2·8980	408gm. beans.
25	1260	17·4630	0·1563	0·1261	0·0302	2·7468	——
,, 26	1150	17·8542	0·1654	0·1220	0·0434	2·6532	——
,, 27	2080	21·8025	0·2218	0·1761	0·0457	3·0784	1700cc. lager beer (5 bottles)
,, 28	1100	19·7281	—	0·1224	—	2·7546	—— *urates.*
,, 29	1030	17·8625	0·1645	0·1250	0·0395	2·6150	——
,, 30	1120	21·5621	0·2475	0·2038	0·0437	3·5430	Usual mixed food.

Except when mentioned, only purin-free fixed diet was taken.

The total urinary purin-N. during two days after the ingestion of 450gms. of chicken was 0·4448, the endogenous purin for two days was 0·3250 (0·1625 × 2), hence 0·1198 was derived from the purin bodies contained in the chicken. As Table IV. shows that these were 0·2200 N., 54·4 per cent. ef the food purin appeared in the urine as exogenous purin.

Five hundred and thirty-seven gms. of plaice contain 0·1707 purin-N., and the total urinary purin was 0·4244. This minus 0·3250 endogenous purin leaves 0·1004, or 58·7 per cent. of exogenous origin. Similarly, 400gms. of beef contain 0·2400 purin-N., the total urinary purin was 0·4378; this less 0·3250 endogenous purin = 0·1138, so that 47·4 per cent. of the beef purin appeared in the urine as exogenous purin.

It thus appears probable, that allowing for the variations which occur in different animals, as well as in separate species, the system excretes in the urine (within 48 hours) about one-half of the fish, fowl or beef purins contained in the food. These figures confirm the results of Burian and Schur as regards the metabolism of beef, and indicate that the purins or alloxur bodies of fish and fowl flesh undergo similar chemical or vito-chemical changes during their passage through the human body.

The Metabolism of Vegetable Purins.

As vegetables contain a high percentage of water, or increase their holding during the processes of cooking, they would appear of less practical import-

ance in respect to their purin contents than meat food. Camerer, however, showed that a vegetable diet increased the urinary alloxuric nitrogen, and von Noorden states that patients on a diet of beans, asparagus and sauerkraut excrete almost the same amount of uric acid as upon a meat diet, and Douglas found that with a diet rich in peas, beans and lentils, the output of uric acid was 0·702gm., as compared with 0·675gm. with ordinary food, and concluded that vegetables poor in proteid diminished, and those rich in proteid increased the uric acid excretion.

The estimations contained in Table VII., show that vegetables known to be rich in proteid matter are also rich in nuclein, and those poor in proteid are also poor in nuclein. Herein, therefore, lies the solution of the hitherto supposed anomaly. Naturally the increase in digestive activity necessitated by the less digestible food may account for a slight rise in the endogenous purin amount, but the remainder is probably due to the purin bodies contained in these foods. To put this conclusion to a practical test, additional experiments were made to determine the exogenous purins after ingestion of haricot beans. The beans, which were in a thoroughly dry condition, were first weighed, allowed to soak in water for twelve hours, then boiled and re-weighed. All the three subjects of the experiment ate from the same lot of beans, and at the same time; otherwise, during the whole experiment, the food consisted of eggs, milk, cheese, bread, butter, sugar and rice. Tables XV., XVI., and XVII give the results obtained:—

Table XV.

W.H., weight 70 kilos, health normal. Urine free from albumen and sugar.

1901	Urine	Table N	Total Purin N	Uric acid N	P_2O_5	Remarks
Oct. 23...	900...	16·8940...	0·1660...	0·1270...	2·620...	——
,, 24...	1150...	17·2368...	0·1857...	0·1391...	2·898...	408gm. dried beans.
,, 25...	1260...	17·4630...	0·1563...	0·1261...	2·746...	——

Table XVI.

N., 49 kilos. Healthy. Urine normal.

1901	Urine	Total Purin Nitrogen	Uric acid Nitrogen	Remarks
Oct. 22...	960...	0·1478...	0·1180...	Purin-free diet.
,, 23...	760...	0·1504...	0·1290...	,,
,, 24...	670...	0·1589...	0·1348...	,, +75—90grms. haricot beans.
,, 25...	560...	0·1095...	0·0979...	,, free diet.
,, 26...	1310...	0·2315...	0·1985...	,, Usual mixed diet, with coffee.

Table XVII.

M., aged 7 years, 22 kilos. Healthy and urine normal.

1901	Urine	Total purin Nitrogen	Uric acid Nitrogen	Remarks
Oct. 22 ...	535 ...	0·1224 ...	0·1013 ...	Purin-free diet.
,, 23 ...	560 ...	0·1170 ...	0·0927 ...	,,
,, 24 ...	570 ...	0·1355 ...	0·1177 ...	,, +120—150grms. beans.
,, 25 ...	650 ...	0·1072 ...	0·0900 ...	,,
,, 26 ...	510 ...	0·1570 ...	0·1463 ...	,, Usual mixed diet.

In each case there was a distinct increase in the urinary purin on the day when the beans were eaten. The fæces from W.H. yielded 0·0407 N. as exogenous purin-N. As 408gms. of haricot beans

contain 0·0920 purin-N., 0·0513 purin-N. was absorbed. The increase of the urinary purin over the normal endogenous factor (0·1625 N.) was 0·0232, and represented 45·5 per cent. of the food purin-N. In the case of N., there was an increase of 0·0090 N. above the average normal endogenous quantity of 0·1250 N., an absorbed amount of bean-purin 0·0162 N., and thus an exogenous urinary purin of 56·2 per cent. With M., there was an increase of 0·0180 N. absorbed, giving an exogenous urinary purin of 55 per cent.

Thus these estimations demonstrate both the presence of purin bodies in the pulses, and their relations with the functional excretions. They furnish an explanation of observations which have hitherto appeared to be improbable, and Table VIII. should therefore be of use in regard to the dieting of patients whose exogenous nuclein metabolism is deficient. At the same time the experiments point to the necessity of exact estimations of the individual metabolic activities.

Haig has recently called attention to the effect of asparagus upon his body-functions. Asparagus contains a considerable quantity of asparagin, and he contends that this substance undergoes easy conversion into uric acid. At any rate, after the ingestion of asparagus, his uric acid excretion showed a considerable rise in quantity, and the symptoms he obtains quickly appeared. He took salicylic acid to remove the uric acid from the circulation, and increased his usual quantity of potatoes, with the object of getting the blood into a

good condition of alkalinity to dissolve the uric acid it contained, and hold it in solution until excreted. These measures were successful, and his uric acid excretion returned to its normal level. If, however, the asparagin was the cause of the augmented uric acid excretion, it is not easy to understand how the addition of potatoes to the diet facilitated its removal. Asparagin is one of the principal extractives contained in potatoes, and hence the added quantity would increase the amount of uric acid. The cause underlying Haig's observations is much nearer to hand. Asparagus contains purin bodies, and the urinary purin is proportionately increased.

The results obtained in the study M. are of particular interest, as I have been unable to find any record of the *endogenous* purin of a child of this age. They show that the processes of cell growth necessitate the formation, and as will be seen later the partial excretion of a greater amount of nuclear substances than prevail in the adult economy, and as no evidence exists that ingested nuclein is built up into cell constituents, it is obvious that the increased excretion is to some extent a measure of the intense vitality which the younger tissues possess, and which enables the production of the highly-complex nuclein from the ingested proteid.

The Metabolism of Beverage Purins.

It has been observed that in the countries where fermented drinks are commonly used, the diseases

A. W. Fuller (*Lancet*, p. 1012, 1903) has recently estimated the purin output of 63 healthy children under reliable and satisfactory dietary conditions. He finds that the purin excretion bears no relation to age, sex, or bodyweight, but that there are marked variations suggesting distinct idiosyncrasies or hereditary tendencies.

due to perverted metabolism are more frequent than in those in which distilled spirits are consumed. Hence in gout and allied conditions, it is customary to forbid beer and porter, and to allow small quantities of whisky and rum. The question as to the effect of alcohol on metabolic processes is, however, not yet fully decided. Hammond, in 1856, found that alcohol diminished the excretion of uric acid and urea after normal, insufficient and excessive diets. Riess, in 1881, gave adult men 3—5gms. of absolute alcohol per kilo-body-weight each morning for 13 days, and observed a diminution of 15—16 per cent. urea and uric acid. Herman, in 1888, obtained no increase in the uric acid excretion after the addition of Burgundy, Bordeaux and Hungarian wines to the usual diet. Herter and Smith, in 1892, pointed out that whisky was without influence on the uric acid elimination. V. Jaksch, in 1894, stated that when alcohol was given to children in acute and chronic illnesses, the uric acid and urea were diminished. Laquer, in 1896, observed a diminution of the total alloxuric nitrogen after large quantities of alcohol. Lebers, in 1897, gave 400—1,000cc. of Malton sherry to several persons upon a fixed solid and liquid diet, and observed a decreased amount of total uric acid nitrogen. v. Noorden, in 1896, reported the case of a patient who took large quantities of alcohol without any alteration in the uric acid excretion.

On the other hand, Herter and Smith found an increase in the uric acid excretion after champagne, and Rosenfeld had a patient whose uric acid rose

considerably after a single bottle of beer. Chittenden observed in dogs fed upon milk crackers and beef, a decided increase in the uric acid (0·028 to 0·0472gms.) after the administration of an average dose of 2·5cc. of absolute alcohol per kilo of body weight, but an important decrease in the total nitrogen output. Donogany und Tibald, in 1895, fed dogs upon a fixed diet, and found that the daily addition of 9—30cc. of absolute alcohol caused increased uric acid excretion. Later researches upon nuclein metabolism in dogs have shown that their liver activity is very pronounced, and that they excrete the greater portion of their nucleins as urea or the intermediate body, allantoin. As alcohol is a well-known cell depressant, a possible explanation may be found in the diminished activities of liver and kidney functions, and the consequent lessened excretion of urea due to the deficiency in the uric acid decomposition, and its resultant increased excretion. In man at first alcohol will in the same way depress cell functions, and lead to a diminished production of uric acid. But as alcohol promotes oxidation, the decreased production, if any, will be small. The decomposition of the uric acid, however, may not be normal, as alcohol in the human subject diminishes the solubility of urates, and thus hinders their transmission to the liver as well as lessens their chances of oxidative katabolism by the depressed zymogenic functions. Hence the elimination of both exogenous and endogenous purins will be delayed or incomplete and their deposition in the tissues favoured.

Chittenden and Beebe (*Amer. J. Phys.*, 9, 1903) state that pure alcohol given with water during fasting leads to decreased uric acid excretion; given with meals leads to increased uric acid excretion, due probably to a disturbed metabolism; the same amount of alcohol given in the form of beer or wine produces more effect, so that there are substances in beer and wine other than alcohol which alter purin elimination.

But if alcohol produces decreased uric acid elimination, how can the pronounced increase after beer and fermented drinks be explained; for the alcohol they contain should diminish the excretion of uric acid, and favour its deposit in crystalline form? The key to the problem may perhaps be found in the fact that each litre of beer contains 0·05 purin nitrogen or 0·12 xanthins, and thus furnishes the precursors of the increased urinary purins. With the view to practically demonstrate this supposition, on a day when no laboratory work was undertaken, I drank $1\frac{2}{3}$ litres of lager beer. Table xiv. shows that the urine for the day amounted to 2080cc., and contained 18·2640gm. total N., 0·2218 total purin-N.,and 0·1761 uric acid-N. In the 1700cc. of beer there would be 0·090 purin-N., and my endogenous purin quotient was 0·1625. As the diet was purin-free, it is reasonable to attribute the increase of 0·0583 N. to substances present in the beer. The experiment, however, was not entirely free from objections. The quantity of fluid was greater than the daily average of 1000cc. milk, and Laquer has shown that after $1\frac{1}{4}$—$1\frac{1}{2}$ litres of water the alloxur N. is increased. Additionally, it is contrary to my usual custom to take alcoholic beverages, and so the effects would be more pronounced; thus, although the general depression of nervous and metabolic functions would diminish the purin excretion, the dilatation of the smaller blood-vessels and capillaries might assist the removal of an abnormal amount of undecomposed trioxypurin. The experiment was therefore repeated in the following manner:—To one litre of lager beer,

1cc. of strong H_2SO_4 was added, in order to preserve the solubility and identity of the purins, the alcohol distilled off, the remainder evaproated to 500cc. and then neutralised. The whole was drunk at 9-0 a.m. on the day of the experiment, and a purin-free diet observed. Table xviii. gives the results obtained.

TABLE XVIII.

	Quantity of urine.	Total Purin nitrogen.	
Nov. 23 ...	1080cc. ...	0·1680 ...	Purin-N.-free diet.
,, 24 ...	1150 ,, ...	0·1590 ...	,, ,,
,, 25 ...	1250 ,, ...	0·1800 ...	,, +1 litre lager beer.
,, 26 ...	990 ,, ...	0·1640 ...	,,

As each litre of beer contains 0·05 purin-N., this second experiment shows an increase of 0·1800 – 0·1625 (normal endogenous factor); therefore 0·0175 or 35 per cent. of exogenous purin is due to the beer purins. The results of the second experiment show that the first yielded, through adverse conditions, too high an amount. The low percentage obtained in the second experiment led to an enquiry as to the form in which the purin bodies exist in beer, and further analyses were made in which the beer was acidified, the proteids removed by lead acetate, and after removal of the lead by H_2SO_4, the free xanthins were determined by the usual process. One litre of lager beer contained 0·0327 free-purin-N. This amount would raise the 35 to 55 per cent., and leave the question of the metabolism of the bound purins of beers to a later investigation.

Wines do not appear to contain any purin bodies,

and their harmful properties in gout must be due to some other constituent. Luff points out that the ethereal constituents neither lessen the solubility of the sodium biurate nor hasten the decomposition of the quadriurate, and considers that certain wines cause alterations in the liver metabolism, and an increased production of glycocin. Others have attributed their effects to their acidity, but the acid wines, clarets, etc., appear the least harmful.

The *methyl-purins*, tea, coffee, and cocoa, increase the total urinary purin, but do not markedly affect the uric acid excretion. Burian and Schur found that 35 per cent. of caffeine ingested reappears in the urine. Bondzynski, Gottlieb and Rost observed that after *caffeine*, one-fourth, and after *theobromine* one-third, appear as methyl-xanthin in the urine. Krüger and Schmidt, in 1901, gave a patient 0·5—1·5gm. of *pure* caffeine per day, and observed that:—

After	50mg.	caffeine,	33·3%	appeared	in	the urine.
„	100 „	„	28·0%	„	„	„
„	200 „	„	19·3%	„	„	„
„	400 „	theobromine,	47·0%	„	„	„

Thus the metabolism of the methyl purins appears to vary with the quantity ingested. The manner in which the methyl group is liberated by the cell protoplasm is said to determine the amount of stimulus which the tissues receive from these substances. The xanthin group, as already remarked, is almost without any excitatory action, and its metabolic end products are constant. Perhaps,

therefore, the variations in the excretion of unchanged methyl-purins is dependent upon the amount of the total reactive energy they invoke.

The Purins of the Fæces.

That the fæces contain nuclein bodies has been long known, but in metabolic experiments it has not been customary to examine them for other than the total nitrogen. Weintraud, in 1895, first called attention to the existence of small quantities of xanthin bodies in fæces. Later in the same year he stated that 0·100 —0·130gms., and in 1896, that 0·100—0·500mg. were present each day. Petrén found 0·0357 and 0·0680gms. per 20gms. of dried material from milk and mixed diets respectively. Umber, on a diet of 500gms. meat, 300gms. bread, 80gms. butter, two eggs and coffee obtained 0·0965 alloxur N. (0·240gms. xanthin), and with 2,000cc. milk, and 100gm. meat 0·1034 alloxur N. (0·288gms. xanthins). Burian and Schur found 0·0100 purin-N. (0·0025 xanthin) on a purin-free diet, and Parker observed 30—38mgms. xanthin on milk, 49—65mgms. on mixed, 60—80mgms. on meat and 71mgms. on carbohydrate diets. If these figures are correct, there exists wide differences in the constituents of fæcal excretions. Probably, however, the methods employed will account for the variations, as Weintraud and Umber used the Krüger-Wulf method, which does not entirely precipitate all the purin bodies, yet carries down other nitrogenous substances. Petrén, after boiling

in 1 per cent. H_2SO_4, removed the acid and albuminous bodies by barium hydrate and ammonium chloride. With this method, it is difficult to wash the precipitate free from carbohydrate substances, and I found it necessary to decompose the silver-purin by H_2S, and again precipitate with $AgNO_3$. Parker determined the purin-quantities in the first precipitate by Salkowski's methods. More convenient is the process used by Burian and Schur, but several modifications are necessary. These are included in the description of the method I employed.

The fæces were dissolved in a 1 per cent. solution of H_2SO_4, dried on a water bath, and reduced to a fine powder, or treated directly with 1 per cent. H_2SO_4. 10—20gms., or the total daily or weekly quantity, were boiled for five to twelve hours in 1000cc. of 1 per cent. H_2SO_4. After neutralisation, filtering, acidifying with acetic acid, reboiling and subsequent filtration, the solution was heated and excess of copper sulphate and sodium bisulphite added. The resultant white-brown precipitate was well washed with water at 60°C., suspended in boiling water, and then decomposed by H_2S or colourless potassium sulphide. The filtrate, after removal of the copper sulphide, etc., was evaporated in acid solution to about 200cc., then made strongly alkaline, filtered, acidified and re-precipitated with excess copper sulphate and sodium bisulphite. The copper sulphide was again removed, the filtrate evaporated to 200cc. in acid solution, made alkaline with NaOH, filtered, the filtrate acidified with acetic

acid and then saturated with ammonia. Finally, ammoniacal silver nitrate solution was added and the nitrogen of the precipitate, after thorough washing with water at 60°C., estimated by Kjeldahl's method. The daily fæces were differentiated by charcoal.

The origin of the fæcal purins is not quite clear. When a purin-free diet is taken, there is still an appreciable quantity present in the fæces. No xanthin substances have been found in the fluids obtained from biliary fistula, and under normal conditions there is probably no excretion of metabolic end-products through the intestinal walls. But when the food is rich in purins, particularly when they exist as undecomposed nucleins, it is quite possible that there may be an unabsorbed residue, whose amount depends upon individual activities of the digestive secretions. Indeed, Corlette has shown that the nitrogen present in a loop of small intestine is chiefly in a higher nitrogenous form than proteid, and consists of nuclein which by its stronger resistance to digestive processes, tends to accumulate. But there is no evidence that the bowel purin contents vary according to general metabolism. Attempts have been made to show that upon a purin-free diet, no free xanthins occur in the fæces, but the albuminous bodies cannot have been sufficiently removed, as I have always found free xanthins present. There must be some source of these bodies, other than the cell-nuclein, said to be shed from the intestinal wall. In a purin estimation of a pig's stomach, I obtained

0·0229 per cent. of total purin-N., and thus 100gms. of intestinal mucous membrane entirely ablated each day would furnish the necessary amount of fæcal purin nitrogen, but it is impossible that such occurs. It is more likely that the demulcent or irritative effects of certain foods determine the amount not only of cell destruction, but also of the quantities of the several digestive secretions. Possibly the pancreatic juice contains a considerable amount of free-purin bodies, as well as undecomposed guanin.

Considerable difficulty thus exists in obtaining a positive average fæcal purin excretion, even upon a purin-free diet, and it has been found that carbohydrate foods induce higher amounts than meats. If, however, approximate figures could be found for an individual upon a fixed purin-free diet, and if this diet was maintained during the ingestion of large quantities of meats, any distinct increase in the excretion might be reasonably attributed to the additional food taken. Table xix. gives the results of an experiment under these conditions.

TABLE XIX.

Fixed diet of eggs, bread, milk, butter, cheese, rice.	Purin nitrogen of fæces.
Purin free	0·0342
,, ...	0·0310
,, +450gms. chicken	0·0840
,, +537 ,, plaice	0·0464
,, +400 ,, beef	0·0617
,, +408 ,, beans	0·0717

Thus when the 450gms. of chicken were eaten, the increase of fæcal purins was 0·0510 N., and so the

total purin-N. of the chicken 0·2200 is reduced to 0·1700 (*vide* Table iv.), and the percentage of urinary purin would be raised to 63·05 per cent. Similarly, the 537gms. of plaice exogenous purin is decreased by 0·0245 N., and its percentage excretion changed to 68·5 per cent; 400 gms. of beef caused an excess of 0·0267 N., and an increase to 53·3 per cent. exogenous urinary purin.

Weintraud has stated that the purin excretion by the fæces is so small as to be neglected, and hence its consideration is omitted from contemporary studies. The above results, however, show that if *large* quantities of purin-rich foods are taken, or the digestive powers are weak or overstained, any quantitative experiments should demonstrate the exact absorption as well as the excretion of nuclein substances.

Recently I have carried out a number of experiments upon the purin bodies of the fæces, in order to determine their relation with the food nucleins and to further elucidate their source. To obtain amounts of purin bodies large enough to separate into their several constituents, the fæces for six days were mixed together and treated by the method previously described. Material has been available from several healthy persons and also patients suffering from rheumatism and gout, and their excreta have been investigated at intervals of three and six months. The results yielded so far, have shown that normally, upon a fixed diet, the purins of the fæces are fairly constant in quantity. Krüger and Schittenhelm found that the excreta of one patient consisted of guanin, adenin, xanthin and hypo-

xanthin, and I can confirm such observations. In the majority of my cases, the purins have been separated and isolated, and the results obtained are useful in pointing towards their source.

When, in addition to the usual diet, measured quantities of guanin, hypoxanthin, xanthin, nucleic acid and thymus have been taken there has been an appreciable increase in the purin bodies of the fæces. Guanin, when taken by the mouth, has increased the guanin yield of the fæces by 50 per cent. Xanthin, thymus and nucleic acid have caused a slight excess, but hypoxanthin has always been well absorbed. The total results will be published later. The purin bodies of the usual meat foods are apparently well absorbed, and it is only when their quantities are in great excess that the purin bodies of the fæces need to be considered in clinical or precise feeding experiments. But there are still many possibilities in regard to their cleavage or oxidation products whilst in the alimentary tract, which further investigations upon the fæces from diarrhœic, enteric, or dysenteric patients may possibly clear up.

These results, together with those of the urinary purins confirm, to some extent, the statement of Burian und Schur that 50 per cent. of the purin contained in the food reappears in the urine. But such amount can only be taken as a broad average, and is applicable only to healthy individuals upon perfectly assimilable diets. Allow such factors to be relatively or absolutely altered, and the necessity is apparent for the determination of personal factors if our knowledge of nuclein metabolism is to be put to

practical use. The whole experiment, nevertheless, shows that fish, fowl and meats all invoke similar reactions in the tissues, and that by the aid of a table containing the "purin-percentage," their quantitative relations to uric acid and the xanthin bases can be determined.

Although it is difficult to obtain nitrogenous equilibrium with great variations of food, Table xx. shows that but little disturbance occurred. The number of eggs eaten each day precluded precise estimations of the food nitrogen, and the figures are therefore taken from König's analyses.

TABLE XX.

	Food N.	Urine N.	Fæces N.
Oct. 13	22·3	20·2132	1·3078
„ 14	21·6	20·4975	1·3078
„ 15	25·4	23·5683	2·1536
„ 16	21·6	20·4680	1·3078
„ 17	21·6	19·4215	1·3078
„ 18	26·1	23·7294	2·4080
„ 19	20·5	19·6536	1·0776
„ 20	20·5	18·2966	1·0776
„ 21	26·3	22·4680	2·1168
„ 22	20·8	18·2990	1·0776
„ 23	17·8	16·8940	1·0776
„ 24	22·4	17·2368	3·8896

THE RATE OF ELIMINATION OF FOOD PURINS.

The rate of elaboration and excretion of the exogenous purins, or, in other words, the minimum time the metabolic organs require to transform and transfer the mono- and di-oxypurin of the food into

the tri-oxypurin of the urine, is of practical as well as of critical interest.

The views of Hopkins and Hope thattheimmediate increase of uric acid after a meal cannot be due to the nucleins of the food, as they are so slowly decomposed, and that the filtrate from digested nuclein does not contain purin bodies, and of Jerome, that any such increase is directly due to the alloxur bodies contained in the food, are so directly opposed, that additional evidence is imperative. If it could be shown that a certain quantity of fed purin was recoverable from the urine within a definite period, and that the usual meats contained much "free" and little "bound" purin, and that the precipitation of nuclein derivatives from the nuclein digest was hindered by the presence of albuminous bodies, the position of the former workers would be less tenable. Should, moreover, the time occupied by the vital oxidation and katabolism of the food-purin be "constant" for healthy adults, then a "time standard" might be established, and variations therefrom serve as indicators of perverted metabolism. A test powder of hypoxanthin might thus be employed in the same way as the test meal and methylene-blue solution are used in the diagnosis of alimentary and urinary diseases.

The analyses contained in Table v. show that the purins of beef, veal, ham, chicken, etc., exist *principally* as free "purins," and that the glandular organs, liver, thymus, etc., also contain an *equally* large amount of unbound purin-bodies. The results

of Tables x. and xi. are also interesting, as they show that the whole of the expected hypoxanthin nitrogen was voided in *four* hours, and that of the uric acid in *eight* hours. Hence my experiments tend to confirm the observations of Jerome, and to indicate the need for further investigation as to the rapidity of purin metabolism. The reason for the difference in elimination-rate between hypoxanthin and uric acid is not clear; perhaps the oxidases or oxidising processes act best upon the mono-oxypurin, or this body enters more rapidly into combination with albuminous substances than the tri-oxypurin, uric acid, and is earlier oxidised.

Additionally, the experiments of Lœwi and Burian und Schur show that the excretion of the bound exogenous nucleins is generally spread over two or more days, and Lœwi has more recently stated that while a small proportion of these bodies is decomposed in the intestine, the major part is directly absorbed. The slowness of their excretion emphasises the greater metabolic efforts they invoke, and suggests their contra-indication in all conditions of deficient metabolism.

The Exogenous Purin Remainder.

We have so far only considered that portion of the food-purin, which after oxidation to higher forms in the tissues or liver, or in both, appears in the urine in quantities representing about half of the amount ingested. We must now therefore ask what is the fate of the other 50 per cent.?

The answer to this question depends upon the particular theory of uric acid formation we decide to adopt. If uric acid is a terminal metabolic product, then this unexcreted balance will be retained within the system, and lead to interstitial or cellular deposits. This supposition has long been the contention of Haig, who apparently considers that uric acid exists in certain foods, and passes therefrom directly into the urine; and that its rate of elimination, together with that of the uric acid of distinct body formation,is determined by the urinary flow, and that its excretion bears a definite relation to the quantity of urea voided. My own experiments show, however, that the volume of urine may vary considerably, and yet the total purin and uric acid excretions remain constant, and that although the urea bears a direct relation to the amount of proteid in the food, the uric acid is entirely independent of it. Thus the urea-uric acid quotient is clinically worthless. Under abnormal conditions retention may occur, but as Haig himself admits the inaccuracy of his estimations of uric acid precursors in meats, and employs unreliable methods for determinations of urinary uric acid, his conclusions can hardly be accepted. The constancy of the endogenous factor in Table xiii., and the ready appearance of the urinary purins after ingestion point to a nuclein metabolism the reverse of sluggish, and permit the conclusion that with ordinary food and normal metabolism, uric acid is not retained.

Upon the theory which regards uric acid as an intermediate metabolic product, a masterly resume

has just been published by Burian und Schur, and it is thus unnecessary to cite the entire literature of the subject. These writers have also shown that when cats and dogs are fed upon uric acid and xanthins, no trace of uric acid can be found in the blood, and only $^1/_{20}$th reappears as urinary purin. Large quantities of allantoin have, however, been met with in their urines. In rabbits glycocoll is said to take the place of allantoin, and $\frac{1}{6}$th only of the ingested purin is excreted as uric acid.

Mendel, Underhill and White have recently obtained a distinct increase of uric acid output in man after feeding with nucleic acid and an increase of allantoin under similar conditions in dogs and cats. Mochizucki found an increase of uric acid excretion in man after the administration of thymus gland per rectum, but Mendel, Underhill and White were unable to confirm this result.

Burian and Schur noted a rise in uric acid excretion after subcutaneous injection of sodium nucleate in dogs, and Kuchman, Mendel, Underhill and White have recently obtained allantoin from the urine of cats and dogs after subcutaneous, intravenous, and intraperitoneal injections of nucleic acid. In many cases, however, toxic symptoms were produced and the animals died. When urates were injected into the systemic and portal circulation a large output of allantoin occurred.

Borrisow, Pohl and Poduschka have lately observed that in dogs hydrazin poisoning leads to increased allantoin excretion. The seat of the allantoin formation is in the liver.

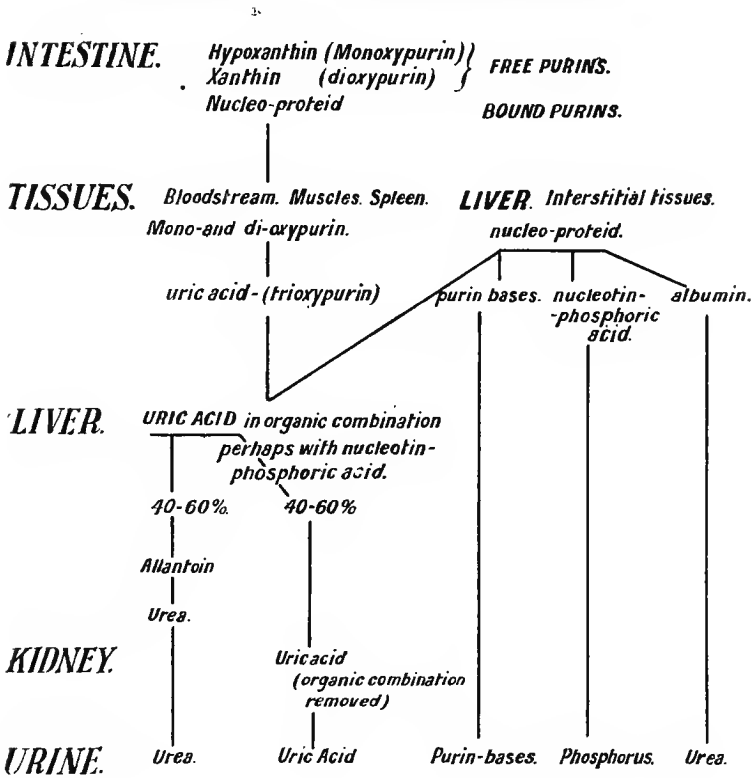
Luzzato (*Zeit. f. Physiolog. Chemie.*, p. 537, 1903) observes that when allantoin is fed to dogs, 70% is excreted unchanged—when fed to rabbits, it is badly absorbed and in part is excreted as oxalic acid. After meat, uric acid and thymus, allantoin is a constant constituent of dog's urine.

Through the courtesy of Prof. Lafayette B. Mendel I am enabled to state the as yet unpublished experiments made by B. White, who finds that intravenous injection of uric acid (in the form of lithium urate) in the cat leads to a distinctly increased excretion of allantoin. When sulphonal was given to dogs with the view of diminishing hepatic metabolism (synthetic and oxidation changes) there was a diminished allantoin excretion after intravenous injections of urates. Similarly in "sulphonalized" dogs the ingestion of large amounts of nucleins (pancreas) was followed by little or no allantoin output.

The autolysis of cellular tissues is accompanied by a marked increase in their purin-base contents. Schmidt-Nielson has described such an occurrence after autolysis of herring flesh, and Pohl found a higher percentage of allantoin in the liver of dogs after autolysis than when the organ was fresh.

Small quantities of allantoin have also been observed in normal human urine, and in the renal secretion and ascitic fluid obtained from cases of liver cirrhosis. Swain considers that allantoin may be looked upon as an intermediary product between uric acid and urea, and that under ordinary circumstances about 50 per cent. of the uric acid produced is almost completely oxidised to urea, but that when the metabolic organs are unable to fully decompose the purin radical, more allantoin and less urea appear in the urine. Hence the variation of end products in the nuclein metabolism of dog, cat and man depends rather upon the extent of circula-

tory and metabolic activity than upon definite peculiarities. We may, therefore, infer that uric acid is now more generally regarded as an intermediary metabolic product, and that its appearance in the urine is due to incomplete decomposition rather than the complete synthesis.



Without committal to the precise details, of which we are still in ignorance, the above diagram probably represents the course of the exogenous purins in the body.

It will be seen that the exogenous nucleins simply pass through the body. Their phosphorus may be retained for synthetic or organic purposes, and the small amount of albumin they contain may be used up in the tissues, but their purin contents are not employed in the synthesis of cell nucleins. Through some decomposition of the purin ring, a definite proportion of the purin bodies are liberated, oxidised and excreted as uric acid, and the remainder eliminated as urea or as bodies intermediate.

Kaufman and Mohr, from recent experiments, have concluded that the extent of this cleavage depends upon the individual and his daily disposition, but the majority of current studies show that the variations lie between 40 and 60 per cent. of the total purin taken with the food, and these constants suggest rather that the constitution of the purin nucleus or polymerisation of the cleavage products play a prominent part.

Soetbeer and Ibrahim have lately published the experiments which led them to conclude that uric acid after ingestion or injection is almost entirely excreted as such by man. According to their figures they obtained 80—98·9 per cent. of the uric acid from the urine after subcutaneous injection of 1·26gm. of uric acid dissolved in piperazine. As will be seen from the following table taken from their

results, the inferences they make are hardly permissible.

Average for 20 previous days	0·3345gm. urinary uric acid nitrogen.
Day of experiment, injection of 0·42gm. uric acid N. ...	0·6739
Following days	0·4106
„ „	0·4518
„ „	0·4283
„ „	0·4283

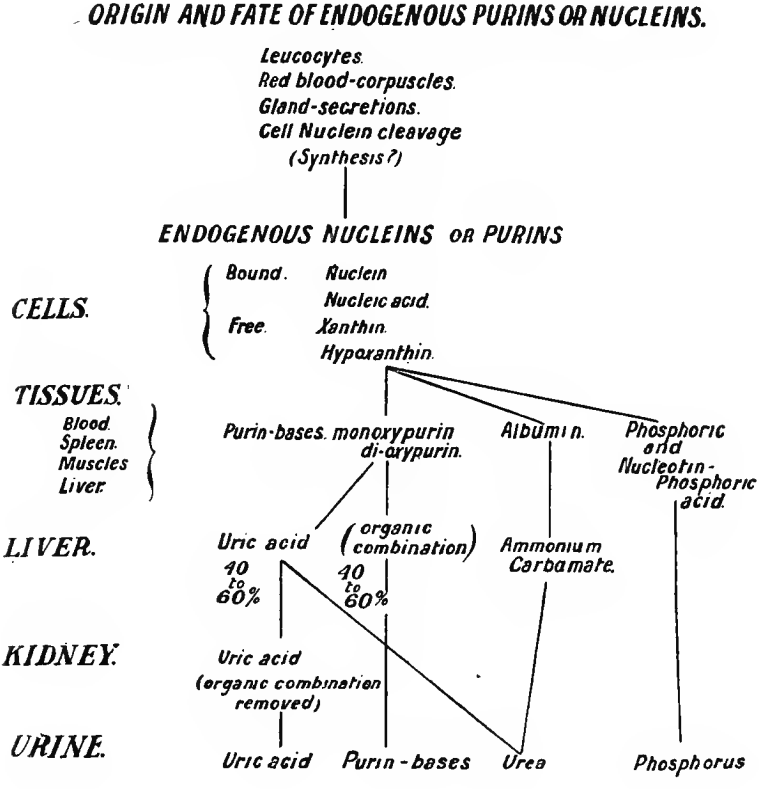
Burian und Schur, discussing these experiments, remark that if the excess of the four following days over the average of the twenty previous days be added to the increased amount upon the day of experiment, then 171 per cent. of the injected uric acid was eliminated. It is probable that the average should be 0·4297gm. uric acid, and then 58·1 per cent. of the injected uric acid would have been excreted as such in the urine. If the average, however, was really 0·3345gm. uric acid N., then it is apparent that the uric acid was decidedly toxic and led to an immediate increase of the endogenous uric acid and this increased excretion continued for several days. The results of Sœtbeer and Ibrahim appear after all to support the previous statement, that 50 per cent. of the exogenous purin bodies are oxidised to uric acid and 50 per cent. are further broken down and excreted as urea or intermediate bodies.

Endogenous Purins.

The sources of endogenous purins are probably numerous and the quantities derived from each may vary with the hourly activities and daily needs. Although our present knowledge upon this point is somewhat inadequate, we may be sure that in pathological conditions alterations in any one of the factors may lead to diminution or increase of endogenous purins. So far as experimental results can suggest normal action one portion of the total endogenous purins is broken down to urea and the remainder excreted as uric acid. Abnormal endogenous purin metabolism may, therefore, consist in an increased production with excessive or diminished destruction, or in decreased production with excessive or diminished destruction. Hence arises the difficulty of any correct inference from the results of endogenous purin elimination. Constancy of endogenous purin excretion points to normal metabolism and the maintenance of the several factors concerned. Variations in the endogenous urinary purin of the same individual upon a fixed diet indicates altered relations of the contributory functions. The table on p. 115 will perhaps give a broad idea of the origin, course and fate of the endogenous purins within the body.

How is the nucleoproteid transformed into the simpler purin bodies? In the laboratory, the processes used for such purpose are lengthy and elaborate, and cleavage products, such as pentose, xylose, hexose, albumin, phosphorus, pyrimidin

derivatives, cytosin, uracil, and nucleotin phosphoric acid are obtained. Is there in the body a direct splitting of nuclein into uric acid, or is uric acid only an end product of the mono- and di-oxyxanthins? In what way is katabolism



affected by pathological conditions? Do ferments play an active part, or are physico-chemical processes more usually employed? These questions suggest the extent of an unexplored and a difficult field.

When we take up the subject of the circulation of purin bodies in the blood-stream and tissues there is more evidence to cite. Without doubt the presence of uric acid can be demonstrated in the blood of patients suffering from certain diseases, but in healthy blood the general tests for the presence of uric acid are not applicable. Substances there exist which interfere with the action of these tests, or the uric acid and xanthin bodies circulate in loose combination with some organic compound. It has been shown that the addition of certain reagents to solutions containing uric acid entirely prevents or markedly hinders the precipitation of the purin bodies, and the suggestion of Minkowski that nucleotin-phosphoric acid forms the medium for their transmission certainly deserves further therapeutical application. After large doses of food purins, or in those conditions in which the purin bodies are present in excess in the blood stream, the administration of certain organic compounds might lead to a loose combination which would favour elimination and prevent uratic infiltration.

It is more than probable that the form in which the purin bodies exist in the tissues is more productive of pathological lesions than the quantitative excess. When one remembers how varied is the composition of the blood in different parts of the

system—here venous, there arterial, here containing much CO_2 and excretory products, there much oxygen and nutrients, it is conceivable that few organic compounds can exist in a permanent form. Hence it is not unlikely that we may ultimately abandon to some extent our conception of bi- and quadri-urates of soda and substitute therefor the thesis of uric acid organic combinations as a necessity for their normal circulation. If we allow such a possibility, then some idea may be gained of the resultant effect of the presence of the imperfect metabolites in the tissues, and our therapeutics be directed to the restoration of normal metabolism and consequent freedom from imperfectly metabolised bodies, rather than to the attempted solution and elimination of uric acid. When uric acid exists in normal combination, it does not irritate the tissues, but if the blood contains abnormal substances and consequent altered osmotic and physico-chemical conditions prevail, necrosis and uratic infiltration frequently result.

A large number of uric acid compounds have been obtained in the laboratory and some of them are isomeric. May not a similar number occur within the body, and perhaps some individual combination be responsible for the particular symptoms and lesions which certain families or persons exhibit?

The seat of the endogenous uric acid formation has been assigned by numerous workers to the spleen, kidney and liver respectively, but Mendel and Jackson consider that in mammals no one organ alone performs such functions. Its destruction, according to

recent work upon the subject, takes place mainly in the liver, but Wiener, working with organ extracts, showed that such prepared from liver, kidneys and muscles were able to decompose uric acid. If his methods were reliable and his experiments correspond to intra-vitam processes, the tissues generally may share in the uric acid destruction. But the rôle of the latter must be small compared with that of the liver, and to this conclusion the later experiments of Burian und Schur distinctly point. Probably, therefore, the exogenous purin-remainder is decomposed chiefly in the liver, perhaps partly in the tissues, and finally excreted as urea, or as bodies intermediate.

The endogenous purin may undergo similar metabolism, and only 50 per cent. of the total quantity appear as such in the urine. Table xxi. may be appended as a contribution to our knowledge of its source of endogenous purin, as it indicates a relation between the body weight and the endogenous purin excretion, and points to the probability that the endogenous purin is the expression of that amount of nuclear or protoplasmic activity necessary for the maintenance of cell functions.

TABLE XXI.

	Age	Weight	Endogenous Urinary Purin	
Infant ...	8 days ...	4·5 kilos...	0·0170 Uric acid-N.	...Mares.
,, ...	3 mths....	5· ,, ...	0·0330 ,, ,,	...Bendix.
,, ...	12 ,, ...	8· ,, ...	0·0350 total purin-N.	...Camerer.
Female...	7 yrs. ...22·	,, ...	0·1200 ,, ,,	...Walker Hall.
,, ...	32 ,, ...48·	,, ...	0·1450 ,, ,,	...Walker Hall.
Male ...	23 ,, ...68·	,, ...	0·1530 ,, ,,	...Burian.
,, ...	32 ,, ...70·	,, ...	0·1625 ,, ,,	...Walker Hall.
,, ...	28 ,, ...77·	,, ...	0·2020 ,, ,,	...Burian.

Burian und Schur in their recent work point out that this table, while indicating a relation between the body weight and the endogenous purin excretion, tells little as to the source of the purin. The origin of tissue nucleins is much more complex. This must be admitted, as so few other figures are at present available, but if further estimations confirm these results, such a relation would make a useful basis for clinical practice.

B. Mendel informs me privately that he has collected a number of new purin estimations, and that his figures correspond, particularly in the case of children, with the above table.

CHAPTER VIII.

THE ROLE OF PURIN BODIES IN MORBID CONDITIONS.

ALTHOUGH healthy blood does not apparently contain uric acid, its presence has been fully demonstrated in the following diseases:—

Pernicious Anæmia. Salomon, "Zeit. für Phys. Chemie.," S. 65, 1878.

Leukæmia. Klemperer, "Untersuchungen über Gicht.," S. 3, 1896.

Anæmia and intestinal inflammation. v. Jaksch, "Deut. Med. Woch.," S. 33, 1890.

Fevers. Malaria, between the attacks. „ S. 33, 1890.
Typhus, after febrile stage. „ „

Gout. Garrod, nature and treatment of gout, 1861.
M. Levy, "Verhand. f. Cong. f. d. Inn. Med.," S. 266, 1896.

Liver. Carcinoma, v. Jaksch.

Nephritis. Garrod, 100cc. blood = 4mg. ur.
v. Jaksch.
Levy, "Virchow's Archiv.," S. 107, 1898, 12 cases.
Klemperer, in uræmic conditions.

Plumbism. Oliver, "Goulstonian lectures," 1891.

Pneumonia. v. Jaksch.

In cardiac, nephritic and pleural exudations it has also been found, and Bouchard identified it in the nasal, pharyngeal, stomach, bronchial, vaginal and uterine mucus and the lachrymal secretions during uræmia.

Numerous estimations of urinary purins have been made in nearly all known pathological conditions, but only in the following have any distinct variations been demonstrated. Nephritis has yielded irregular results: —

PATHOLOGICAL INCREASES OF URINARY PURINS.

URIC ACID:

Alcoholism with enlarged liver. Strauss, "Zeit. für klin. Med.," p. 319, vol. 31.

Carbon monoxide poisoning. Münzer, "Deutsch. Archiv. f. klin. Med.," p. 236, vol. 52.

Cirrhoses of liver. v. Noorden, "Lehrbuch," p. 288. Also in a case of acute yellow atrophy.

Gout. M. Levy, "Berlin. klin. Woch.," p. 389, 1896, during and immediately after acute attacks.

Leucocythæmia. Ranke and many others.

Neurasthenia and Migraine. "His W., Verhand-Cong. f. inn. Med.," 1896.

Pneumonia. Herter and Smith, "New York Med. Journ.," 1892.

Sepsis. v. Jaksch, "Cbl. f. inn. Med.," p. 188, 1896.

Scurvy. v. Jaksch, "Cbl. f. inn. Med." p. 188, 1896.

XANTHINS:

Adipositas. Schreiber und Waldvogel, "Arch. f. Exp. Path. und Pharm.," 42, S. 74, 1895.

Diabetes. Baginsky und Sommerfeld, "Zeit. für Phys. Chemie., S. 412, 1895.

Diphtheria. Baginsky und Sommerfeld, "Zeit. für Phys. Chemie.," S. 412, 1895.

Scarlet fever. Baginsky und Sommerfeld, "Zeit. für Phys. Chemie.," S. 412, 1895.

Nephritis. Kolisch and Tandler, "Stuttgart," 1895.
Croftan, "New York Med. Journ.," Aug. 11, 1900.

Thyroidea. Schreiber und Waldvogel.

PATHOLOGICAL DECREASES IN URINARY PURINS.

URIC ACID:

Anæmias. Brandenburg, "Berlin klin. Woch.," S. 137, 1896.
Honigman, "Cbl. f. inn. Med.," S. 873, 1897.
Taylor, " " " "
Lœwy, "Cbl. f. inn. Med.," S. 188, 1896.

Gout. Decrease 1—3 days previous to attack. His (l.c.) Vogt. S. (general decrease) "Deutsche Archiv. klin. Med., S. 21, 1901.

Dependent upon the extent of diuresis, variations occur in cardiac diseases, and in epilepsy and chorea. In diabetes, any increase is usually the result of excessive intake of meat purins. In certain fevers, leucopenia, phthisis, and the intermediate stages of gout, the uric acid excretion is normal.

These variations result from the balancing of production and destruction, or of production and excretion. Unfortunately, the great mass of statistics available for reference supply little distinct and

decisive evidence; for until recently, diet was not considered in relation to the results obtained, and although the newer experiments give details of the food taken, there exist but few investigations in which the purin contents of the dietary have been estimated, and the reaction they incite, seriously recognised. It is even now generally thought that in gout the exogenous uric acid formation and excretion are normal, except before and after the acute attacks, and Watson has demonstrated the fact by feeding a gouty patient upon thymus and nucleic acid, obtaining an increased uric acid elimination, and inferring that the nuclein metabolism was normal. The average daily uric acid was 0·307gm. Thymus contains about 0·4 per cent. purin-N., of which one-fourth reappears in the urine as uric acid. On January 6th and 7th, 280 gms. of thymus were given, and the total uric acid excretion was 0·730, or 0·242 uric acid nitrogen; 280gms. of thymus contain 1·12gms. purin-N., of which 0.28 may be expected to appear in the urine as such. If we take the average excretion of 0·307, the total for the two days would be 0·614 uric acid, or 0·205 uric acid N., and the difference between $0·242 - 0·205 = 0·037$ might be considered as due to the thymus. But 280gms. of thymus yield 0·28 exogenous urinary purin. Similarly, on January 9th, 252gms. of thymus were administered, and the uric acid was still only slightly increased. There is no record of the total purin nitrogen or the xanthin bases, so that one cannot draw the conclusions desired, but if the many investigations upon the effect of thymus upon meta-

bolism are correct, it is improbable that the patients' purin metabolism was normal, and possible that considerable retention occurred.

We can now, however, by aid of the average purin percentages of Tables iv.—viii., and the knowledge of the metabolic results recorded in Table xiv., deduct the exogenous portion from the total urinary purin, and so obtain the endogenous factor, or by the observations of a diet containing only purin-free foods, directly estimate the endogenous purin. As we may thus definitely assign some portion of the urinary purin to the presence of purins in food, we may, as the results of this investigation demonstrate, control the individual nuclein metabolism in its relation to purin ingestion by prescribing a dietary based upon quantitative purin estimations. Hence, so far as the reactive processes are concerned, the practitioner should now be able to precisely spare or stimulate his patient's metabolic powers, and under both conditions prognose the character and the intensity of the endogenous metabolism. To this end, indeed, further studies must now be directed. The experiments herein described have shown the importance of diet in its relation to uric acid, and the ease with which we may arrive at the amount of uric acid produced by the body itself, so that the future determinations of urinary purins should have for their object the attainment of definite factors for the endogenous purins, a knowledge of the circumstances that affect their variation, and the acquisition of data as to the rapidity and completeness of the individual exogenous purin metabolism. New estimations of

the purin excretion in many diseases are by these conclusions rendered absolutely necessary, and the results therefrom should lead to more accurate conceptions of those metabolic changes, compensatory or otherwise, which probably accompany all pathological processes. The tendency to ascribe to uric acid a causative influence in the majority of the known diseases may be attributed to an over-enthusiasm, which has sadly enough induced much apathy upon any subject connected with uric acid, and consequently led many to under estimate its rôle in the economy. When, however, pathological chemistry becomes gradually narrowed to the appreciation of cell-reactions, it may direct attention to the nuclear changes as the expression of the dominating factor in cell life, and compel us to regard the endogenous purins as the more important indicator of metabolic processes, and the decomposition of proteids as dependent upon the energy liberated by the cleavage of cell-nucleins.

Kaufmann and Mohr have recently published some estimations of purely endogenous purins in certain pathological conditions. One cannot, of course, compare them with similar results obtained from healthy persons and make conclusions therefrom, but the figures are exceedingly useful as the first of a new series. The following are a few typical cases:—

Sex	Age	Weight	Total Urinary Purin N	Disease
Female	17 yrs	41kg.	0·137	Scarlet Fever
,,	18 ,,	49kg.	0·181	Hæmatemesis
,,	20 ,,	47kg.	0·115	Gastric Ulcer
Male	21 ,,	54kg.	0·213	Perityphlitis
,,	23 ,,	62kg.	0·200	Abdominal Pain
,,	36 ,,	64kg.	0·190	Gastric Catarrh

In gout the same observers and also Vogt conducted metabolic experiments and found an increase of endogenous purins immediately before and during the acute attack. During the intervals the excretion approached the normal. It is, however, exceedingly difficult to make satisfactory deductions from the excretions of different patients. How far these statements may be applied to individual cases is not at present known! The general inference that in gout there is little retention except during the acute attacks is permissible, but accurate figures are only possible by comparison of the excretions of the same individual at varied intervals and under altered circumstances. For the formation of tophi, marked urate retention is unnecessary, as these accretions are only formed slowly and are most probably due to local rather than general lesions. When the kidney is involved, the uric acid excretion is slightly altered, but it is in no sense comparable with the marked variations of the urea and albumin which occur. Exogenous purins are, as a rule, badly metabolised by gouty individuals. This has been recently observed in a case by Reach. It will be shown later that uric acid cannot be "washed out" of the system. Substances that solute uric acid in

the test tube are of little or no use in increasing the output of uric acid in the body. When drugs do increase or diminish the uric acid excretion, they act by directly affecting the cellular processes of the body and not by dissolving out the uric acid deposits.

In leucocythæmia the formation of endogenous purin is excessive and the urine contains very large quantities of uric acid. In this case the endogenous purins are probably diminished by the anæmia and its consequent tissue malnutrition, and augmented by the formation and destruction of enormous numbers of leucocytes.

The increase of uric acid in cirrhoses of the liver is somewhat variable and new estimations are required. Here it is probable that there is decreased destruction of uric acid in the liver.

In neurasthenia and migraine, excess in uric acid elimination has been observed. Whether this betokens loss of nervous control or irregular nervous stimulation is not clear. In either case there is room for extended enquiry in this direction, and results obtained by the use of recognised methods should be useful as regards the prognosis of both the metabolism and the nerve lesions of the patient.

Still, at the moment, the source and function of the endogenous purin are not clearly delineated. A small portion may arise from leucocytic destruction, but distinct evidence of any other origin, although at present unavailable, will doubtless soon be furnished. Apart from theoretical considerations it is certain that diseases of perverted metabolism are frequently accompanied by abnormally increased or decreased

excretion of urinary purin, and just as the secret of successful treatment in tuberculosis depends on its early recognition, so the radical therapeutics of metabolic diseases will depend upon our better knowledge of their earlier stages. Let the now fashionable precepts of individuality in treatment have further application in the practical recognition of a personal factor in the normal and compensatory chemical processes which occur in the human body, and the necessity for lifelong studies of patients and their families becomes at once apparent. Who is to obtain the information as to the metabolism of children of gouty parents and its alterations during their growth? The laboratory worker is unable to make material advances along this line, for the cases he is able to investigate are principally those of healthy, abstemious adults, with perhaps a sprinkling of kindly disposed patients. It is the clinician who alone can furnish the necessary information and statistics. If one be permitted to further indicate the lines of such research, I would remark that opportunities to estimate a patient's endogenous purin, and occasions for systematic determinations of these factors from year to year in children of certain diatheses, must occur to many practitioners. The accumulation of such statistics would not only contribute to our general knowledge of these processes, but would often throw much light upon local conditions.

The occurrence of abnormal quantities of the xanthin bases in the urines of nephritic and gouty patients has been pointed out by Kolisch and others,

but their results were obtained by the (for this purpose) inappropriate Krüger-Wulff method, and as His and Schmoll have failed to find any such increases in gout, additional work is necessary before the theory of the causation of nephritis by alloxur bodies can be accepted. The opponents of the theory are not, however, justified in their denial of its probability. Already cited facts certainly show that in rabbits, unaccustomed to much purin in their foods, degenerative tissue changes occur, and normal growth is hindered. Whether these are due to direct toxic action, or to cellular exhaustion consequent upon overwork, is a matter not yet decided. But Mitchell-Bruce and Bier are both agreed that metabolic perversion throws extra strain upon the vascular and excretory organs, and as a result of some chemical irritant, the heart hypertrophies and possibly the kidney degenerates. The relation, if any, between such excitant and the food purins, is a subject which I hope to further investigate.

Croftan has recently published an interesting paper upon the role of uric acid in morbid conditions. He points out that the accumulation of uric acid in the tissues is simply one of the symptoms of gout, and must not be taken as its cause. The intravenous injection of uric acid into animals caused immediate intra-cellular changes and the excretion of 80—90 per cent. of the uric acid in the urine. He also examined the uric acid destroying quotient of the liver, kidney, muscle, blood and spleen after the removal of these tissues from the body. The human kidney appeared to destroy more uric acid than the

liver, and the muscles more than either the liver or kidney. The presence of salicylates and alkalies accelerated the destruction. He ascribes these results to the action of unorganised soluble ferments, and considers that his experiments favour the renal theory of gout and that the relation of obesity, gout and diabetes, arises from the fact that the organs which destroy uric acid equally catabolise fat and carbohydrates. The association of oxaluria with diminished uric acid excretion, and the presence of these conditions in gout is also dealt with. There is no doubt that Croftan's experiments are ingenious and important, and differ from other workers in the inclusion of human organs; it is at the moment difficult to apply the figures, as we do not know much of the changes which occur during the autolysis of glandular organs and in what ways the available ferments can vary their usual actions.

CHAPTER IX.

The Action of Drugs upon the Elimination of Purin Bodies.

The view which regards uric acid as the actual and only *materies morbi* in gout and allied disorders, still largely prevails; consequently, much therapy is directed against this necessary result of nuclein metabolism. Fashion has decreed the use of numerous drugs in the active and prophylactic treatment of these conditions and the value of each medicament has been measured in terms of its solvent powers for uric acid in the test-tube, quite regardless of the fact that it could not be safely introduced into the blood in sufficient quantities to exercise its soluting properties. The following table shows a few of the results obtained by the use of these much vaunted remedies.

Bain, W. "Brit. Med. Journal," p. 243, 1901.
Bohland, K. "Münch. Med. Woch.," s. 505, 1899.
Goodbody, F. W. "Journal of Physiology, p. 414, 1899.
Good, C. "American Journal of Med. Sci.," p. 274, 1903.
Huber and Lichenstein. "Berlin. Klin. Woch.," No. 28, 1902.
Hupfer, F. "Zeit. f. Phys. Chemie.," s. 303. Bd. 37.
Kumagawa. "Virchow's Archiv," s. 192. Bd. 113.
Laquer, B. "Verhand f. d. 14 Cong. Inn. Med.," s. 333, 1896.
Leber, H. "Berliner Klin. Woch., s. 957, 1897.
Lewandowsky, M. "Zeit. f. Klin. Med., s. 202, 1900.
Salkowski. "Virchow's Archiv, s. 573. Bd. 117.
Schrieber und Waldvogel. "Arch. f. Exp. Path.," s. 69. 42.
Singer, H. "Arch. f. d. Ges. Phys.," s. 527. Bd. 84. 1901.
Weiss, J. "Zeit. f. Phys. Chemie.," s. 216. Bd. 27. 1899.

	Observer.	Duration of Experiment.	Diet.	URIC ACID.		Remarks.
				Average Increase.	Average Diminution.	
Water	Laquer ..	?	?	10%	—	
Alkalies	,, ..	?	?	—	0·020 gm	
Homburg Water ..	Lebers ..	6 days	Fixed mixed	0·173 gm	—	three days later decrease of 0·109 gm
Sodium Acetate 10 gm	Salkowski ..	—	?	—	0·285 gm	supposed diminished formation
,, ,, 13 gm	Kumagawa ..	—	Fixed mixed	0·054 gm	—	in dogs
,, ,, 16 gm	,, ..	—	Fixed mixed	0·064 gm	—	
,, Benzoate 3 gm	Schreiber ..	3 days	Fixed mixed	—	—	
,, ,, 5 gm	Lewandowsky	2 days	Hospital diet	—	—	
,, ,, 7 gm	,,	5 days	Hospital diet	0·032 gm	—	
,, Salicylate 3 gm	Ulrici ..	3 days	Fixed mixed	—	0·049 gm	nitrogenous equilibrium maintained
,, ,, 3 gm	Schreiber ..	4 days	?	0·252 gm	—	
,, ,, 3 gm	,, ..	3 days	Vegetable diet	0·097 gm	—	
,, ,, 4 gm	,, ..	3 days	Vegetable diet	0·140 gm	—	
,, ,, 1 gm	Goodbody ..	4 days	Fixed mixed	0·065 gm	—	
,, ,, 2 gm	,, ..	9 days	Fixed mixed	0·078 gm	—	
,, ,, 2 gm	,, ..	9 days	Fixed mixed	0·250 gm	—	
,, ,, 5 gm	Lewandowsky	3 days	Hospital diet	0·207 gm	—	
Lithium	Good ..	—	—	—	—	without any influence
,, Benzoate 45 grs	Bain ..	4 days	Fixed meat	0·017 gm	—	
Acid Tannic .. 3 gm	Boland ..	4 days	?	—	0·287 gm	
,, ,, .. 3 gm	Ulrici ..	3 days	Fixed meat	0·067 gm	—	nitrogenous equilibrium
,, ,, .. 6 gm	,, ..	6 days	—	—	0·298 gm	
,, Gallic .. 8 gm	,, ..	3 days	Fixed meat	—	0·049 gm	nitrogenous equilibrium
,, Benzoate 8 gm	,, ..	3 days	Fixed meat	—	0·049 gm	nitrogenous equilibrium
,, Quinic .. 8 gm	,, ..	3 days	Fixed meat	—	—	nitrogenous equilibrium
,, ,, .. 6 gm	Lewandowsky	4 days	Hospital diet	—	0·045 gm	
,, ,, .. 4 gm	Hupfer ..	4 days	Fixed mixed	0·019 gm	—	
Aspirin .. .. 3 gm	Singer ..	2 days	?	0·273 gm	—	big fall afterwards
Atropin .. 2 mgm	Bohland ..	3 days	?	—	0·148 gm	
Colchi-sal 9 capsules	Bain ..	4 days	Fixed meat	—	0·013 gm	aromatic sulphate high
Lysidine .. 45 grains	,, ..	4 days	Fixed meat	0·110 gm	—	
,, .. 1 gm	Goodbody ..	4 days	Fixed meat	—	—	
,, .. 2 gm	,, ..	4 days	Fixed meat	—	—	
,, .. 1 gm	,, ..	4 days	Fixed meat	0·018 gm	—	
Piperidin, 30 grains Tartrate	Bain ..	4 days	Fixed meat	0.017 gm	—	slight rise afterwards
Piperazin 45 grains	,, ..	4 days	Fixed meat	0·064 gm	—	aromatic sulphate diminished
Sidonal 75 grains Piperazine quinate	,, ..	4 days	Fixed meat	0·126 gm	—	
Sidonal, Neu. 10 gm Quinic anhydride	Huber ..	5 days	Fixed meat	—	0·116 gm	
Urosine	Weiss ..	—	—	—	—	said to diminish uric acid excretion
Urotropine 30 grains	Bain ..	4 days	Fixed meat	0·03 gm	—	

A glance at the above figures shows that the uric acid output is only slightly affected by drug administration. If we take 0·3750gm. as an average uric acid excretion upon purin-free food, then upon a mixed dietary the average output may be anything from 0·9 to 1·2 grms., dependent upon the amount of exogenous food-purin. As the bulk of the above experiments were conducted upon patients taking meat, the variations recorded are quite insignificant. Only salicylate of soda yielded a distinct increase.

Any excess or diminution in the urinary uric acid may be due to alterations in the amount destroyed by the liver, and it is at present beyond our knowledge to assign the exact cause of these variations, so that when the food contains large amounts of uric acid yielding bodies the question is extremely complicated. Thus, although some of the above experiments were conducted under the conditions of nitrogenous equilibrium, until similar experiments upon the same individuals taking purin-free food are available, it is impossible to make any deductions as to the causes resulting in the slight variations recorded.

In the case of sodium salicylate, however, Schreiber gives the figures obtained from an experiment in which the subject took vegetable food only, and I have nearly ready for publication a similar case in which the patient was fed upon purin-free food and in which the purin excretion was increased about 50 per cent. after the administration of sodium salicylate. Six months earlier the same patient had taken 45 grains of sodium salicylate each day and a similar increase of uric acid output occurred,

although on the days selected for comparison there was a temperature of 99—100°F. It appears, therefore, that when sodium salicylate is administered to a patient upon purin-free food, the endogenous purin or uric acid is at once increased by about 50 per cent., and upon its cessation the uric acid immediately becomes normal. In those experiments in which nitrogenous metabolism was maintained, some observers found a distinct urea increase, and concluded therefrom that the drug led to considerable disturbance of the nitrogenous functions and to increased leucocytosis. When, however, we eliminate the exogenous purin from the question, it is extremely improbable that the leucocytosis or increased tissue destruction would account for the augmented uric acid output. Although further experimental proofs are necessary, it is quite conceivable that this excess purin excretion is due to a synthetic formation and not to any retained products or ordinary cell processes. Luff has already arrived at the same conclusion from a different standpoint; his thesis as to the union of glycocine and urea in the kidney is as yet unproved. The liver is the more likely organ. But his inference that sodium salicylate increases the amount of circulating purin bodies and hence is contra-indicated in gout and for patients with uratic deposits deserves wider appreciation. One thing is certain—salicylate of soda does not wash out retained uric acid and it cannot be employed for such a purpose.

Tannic and quinic acids appear to reduce the purin output, but in the cases recorded above a mixed diet was employed, and it is therefore impossible to con-

Tattersall and Gies (*Amer. Journal of Phys.*, 9, 1903) find that quinic acid does not cause any increase of uric acid excretion in dogs.

clude whether the diminution was due to an increased destruction of the exogenous moiety (particularly the 50 per cent. which is ordinarily excreted as purin bodies), or to a decreased formation of endogenous purin from a depressant action of the drugs; or whether it arose from an abnormal destruction of the endogenous purin moiety which generally escapes further cleavage. The entire question is exceedingly complicated and contains little promise of early solution. Certainly, the same experiments repeated with purin-free instead of a mixed diet, would serve for useful comparison, but in our present state of knowledge one fact stands out prominently, viz., that we have not a drug that can be administered in sufficient quantities to affect the circulation of urates in the tissues, and therefore that such medication is entirely useless.

We are thus, at present, in the condition of waiting for estimations of the endogenous uric acid output of a large number of healthy and diseased individuals. The figures which allow a consideration of the relation of leucocytosis, pyrexia and toxications to nuclein cleavage and excretion are comparatively few. We possess results which point to an excess of urate excretion in pneumonia, anæmia, cirrhoses of the liver, malignant tumours, and the acute stages of gout, but their appraisement is difficult, because of the conditions under which they were obtained. Until more reliable results exist, empiricism and fashion must direct the prophylactic treatment in metabolic disorders. Recent researches have revealed much of the meta-

bolism of cats and dogs, but comparative studies can only point a guiding line to human investigations. If it can be realised that the enormous difficulties of research upon the metabolic functions are being gradually surmounted, and that the study of the cleavage products of the several elements of protoplasm is making rapid progress, it will be then understood that in the extension of our knowledge of the anomalies of body chemistry there are possibilities near ahead of enormous practical importance. It may be conceded that each individual has the capacity of very elastic adaptation, as, for instance, in the conditions of alkaptonuria, cystinuria, etc., but at the same time if the average action of the tissues is determined it may appear that in the majority of circumstances the adaptation follows certain fairly defined courses.

It would seem that the hepatic functions are primarily concerned in gout and the metabolic disorders. During the intervals of the gouty attacks the uric acid excretion is apparently normal, although if any irritated or necrosing tissues exist, slow uratic infiltration is possible, but in the acute attacks there is a slight diminution before, and an excess during the attack. Here again must the question be put, is this increased formation or decreased destruction only?

The consideration of these matters may therefore lead us to the acceptance of a thesis that uric acid is rather the symptom of, than the precise *materies morbi* in gout, and that its effect upon the tissues results rather from its combination with the products

of abnormal metabolism than from its excess in its usual compounds. If we abandon the former views and admit the value of uric acid estimations from the standpoint of symptomatology, we are a step nearer to the cause or number of causes which produce the varied excretions. Thus the study of irregular, hereditary and acquired gout would probably yield a series of uric acid variations quite distinct in character and quantities. Perhaps such results would direct attention to the associations of diet, habits or individual tendencies, and the relations of other metabolic disorders, such as diabetes and obesity.

Until such time as we can obtain more figures for comparison and as a basis for further investigation, in our ignorance of the exact causes of the toxic origin of the gouty diathesis, the only avenue available for actual treatment is the one so often advocated, viz., the maintenance of good digestive faculties, the diminution of intestinal fermentation and putrefaction by suitable remedies, the regular evacuation of the bowels, and the careful control of the circulatory system. The presence of unknown metabolites is very quickly reflected in the action of the heart and vessels—either through an effort of enforced elimination or by their direct effect on the vessel walls. When the excessive urate excretion betokens hepatic insufficiency the establishment of a correct circulatory uric acid combination and hepatic stimulation are indicated.

And thus we arrive at the conclusion that those drugs, which stimulate the hepatic functions or

diminish the variety and extent of abnormal products in the portal bloodstream, best assist the nuclein metabolism of the body and the normal expulsion of its cleavage products. Thus Luff finds that guaiacum is a useful prophylactic in gout, because it is a good hepatic stimulant. Hence the elimination of purin bodies cannot be directly influenced by drugs, although indirectly the excretion of uric acid may be altered, as, for instance, alkalies which stimulate diuresis and large quantities of liquids which demand early excretion, form media in which the purin bodies may be removed from the tissues. The maintenance of the liver functions should nevertheless command the first attention, and hepatic stimulants are the safest means of promoting purin excretion. If the liver is also assisted by the preservation of normal intestinal conditions and is so protected from the toxic products of proteid putrefaction or carbohydrate fermentation, then the circulation of purins in the bloodstream and their excretion through the kidney will be accelerated.

The necessity for systematic estimations of the uric acid and purin excretion of individual patients becomes more important when we regard purin substances as symptomatic. As each person presents variations in both exogenous and endogenous excretions, it will be apparent that for clinical purposes uric acid and purin estimations should be comparative as regards the same individual and not in relation to other patients.

There are some drugs which appear to definitely hinder the elimination of purin bodies. Quinic

acid and its compounds produce a diminished excretion of uric acid in some cases, but their action is slight as well as variable. Sodium salts, as Luff points out, lead to distinct delay, and almost to a retention of purin compounds, as uric acid readily forms insoluble combinations with sodium, and so uratic deposition is favoured.

An interesting outlook in relation to this question is the action of some bodies which hinder or prevent the precipitation of uric acid and xanthin bodies from their solutions. Minkowski some time since remarked upon this property of nucleotin phosphoric acid, and has administered this substance to patients with the view of maintaining the circulating purins in solution and preventing uratic infiltration. At present, however, nucleotin phosphoric acid is not easily obtainable and very few results are recorded.

CHAPTER X.

The Estimation of Urinary Purins.

Although uric acid may be considered more as one of the normal excretions than an extensive factor in the causation of disease, there are many conditions in which a knowledge of a patient's nuclein metabolism is of practical as well as of theoretical value. Recent investigations have shown that a period of diminished uric acid excretion precedes acute attacks of gout, and if such alterations could be rapidly and easily observed, preventive measures might palliate if not avert the attack. The value of individual metabolic quotients has been already emphasised, and the possibility expressed that such information might lead to the earlier recognition of progressive changes in the chemical activities of the liver. This would at the same time indicate the possibility of any calculous deposits. Such evidence would also assist in the elucidation of the sources of endogenous purin, and of those conditions which excite or depress the synthesis and katabolism of nucleins.

The estimation of uric acid is not difficult, but it requires considerable care and attention, a certain amount of apparatus and much more time than the clinical assistant or general practitioner is able to

bestow. The reliable methods which may be used are as follows:—

1. *Ludwig-Salkowski*—precipitation and removal of phosphates—precipitation of total purins as silver-magnesium salt—decomposition by H_2S or potassium sulphide—acidification and evaporation of the filtrate, crystallisation of the uric acid—drying, weighing, or estimation of the N. by Kjeldahl's process.

2. *Hopkins.* Saturation of 100cc. urine with 30grms. NH_4Cl, after 1—2 hours, ppt., washed, acidified and heated to 90°C, evaporated to 30cc. and the uric acid crystallised, dried and weighed.

Modifications:—

Ritter, G. After solution of the ammonium urate, the addition of 20cc. strong H_2SO_4, and titration whilst warm against $^1/_{20}$ normal $KMnO_4$. (1·578 $KMnO_4$ in 1 litre distilled H_2O) 1cc. = ·00375gm. uric acid.

Wörner E. During precipitation the urine heated to 30—40°C, the ppt. washed with hot 10 per cent. $(NH_4)_2\ SO_4$ solution, the ammonium urate dissolved in 1—2 per cent. NaOH solution, and heated on a waterbath until all NH_3 is driven off, and the N. of the evaporated solution then estimated by Kjeldahl's method.

Lewandowsky, M., first ascertains by titration the acidity of the urine, and then makes it nearly neutral. In acid urines the formation of the ammonium urate is slow and imperfect.

Folin und Shaffer employ a solution containing 500gm. $(NH_4)_2\ SO_4$, 5gm. uranium acetate, 60cc.

10 per cent. acetic acid, 650cc. distilled water; 75cc. of this solution are added to 300cc. of urine, 10cc. NH_4OH added, and the whole allowed to stand until the next day. The ppt. is washed with 10 per cent. $(NH_4)_2\ SO_4$ solution, then dissolved in 100cc. H_2O, 15cc. H_2SO_4 added, and the 115cc. titrated against $KMnO_4$.

Dimmock and Branson. 100cc. of urine, warmed to 40°C., saturated with 31gms. NH_4Cl, shaken in a stoppered measuring glass and allowed to stand two to twelve hours. Precipitate washed with dilute ammonia (1—1000), and then placed in 25cc. solution of sodium hypobromite iu a generating bottle and the gas evolved measured in a graduated azotometer. The results are empirical but the error should not exceed three per cent.

Jolles, A., after formation of the ammonium urate, removes the ammonia by heating with magnesia for 45 minutes, then adds 10cc. 1·4 per cent. H_2SO_4, and oxidises the uric acid to urea by $KMnO_4$. To the urea solution NaOH and bromine are added, and the N. evolved collected in an azotometer.

All these methods are, however, too laborious for clinical use. As we have previously seen, both uric acid and the xanthin bases are the result of an undestroyed or unoxidised portion of the total amount of purin bodies dealt with by the system. If food substances are taken that contain methyl-purins, only the xanthin portion of the urinary purins is increased, and if they are absent from the dietary the quantity of xanthin bases in the urine is very small. If no methyl xanthins are present, the total

purin-N represents the purin end products of nuclein metabolism more fully than the uric acid alone. Hence, although the estimations of endogenous uric acid are valuable, much more so are those of the total urinary purins.

If, then, some method for estimating the total purins were devised, which would occupy little time and yet give approximately accurate results, it would be of much practical use. The methods of Haycraft, Herman and Camerer for the estimation of the total alloxur bases offer no easier means to this end. The silver-magnesium precipitate is gelatinous in character, falls only slowly, and takes many days to become firm enough to occupy a constant space. In a long glass tube of $1\frac{1}{2}$cm. diameter, a magnesium-silver precipitate from 100cc. urine

After 12 hours filled 27·5c.m.
„ 36 „ „ 21·5 „
„ 64 „ „ 20·5 „

A small quantity of magnesium silicate was then added, and in 12 hours the precipitate had fallen to 18·4cm., and there remained constant. With a graduated measure, a similar precipitate from 50cc. urine gave: —

After 24 hours 21cc.
„ 48 „ 20 „

Magnesium silicate was then added, and in 10 minutes the precipitate had sunk to 15cc.

The solution was next well shaken, and

15 minutes later the precipitate = 17cc.
24 hours ,, ,, ,, = 20 ,,

at which point it became constant. *Many* similar experiences yielded equal results; 0·01 uric acid was precipitated by Camerer's method and $\frac{1}{2}$grm. talc added.

12 hours later the precipitate = 35 cc.
24 ,, ,, ,, ,, = 33 ,,
48 ,, ,, ,, ,, = 32 ,,
64 ,, ,, ,, ,, = $31\frac{1}{2}$,,
88 ,, ,, ,, ,, = 31 ,,
112 ,, ,, ,, ,, = $30\frac{3}{4}$,,
136 ,, ,, ,, ,, = $30\frac{1}{2}$,,
178 ,, ,, ,, ,, = 30 ,,
202 ,, ,, ,, ,, = $29\frac{1}{2}$,,

Oct. 18.....0·01 uric acid 24 hours after precipitation = 34 cc.
,, 20.....0·01 ,, 24 ,, ,, = 34 ,,
,, 21.....0·01 ,, 24 ,, ,, = $34\frac{1}{4}$,,

It would thus appear that it is possible to obtain the silver-magnesium-purin precipitate as a firm, constant mass, as well as in gelatinous form. Should later experiments confirm this conclusion, it might be practicable to determine a specific value for each cc. of the precipitate, and so calculate from its amount the approximate quantity of purins present in the urine. But the reagent which serves as a medium to produce this result must itself occupy a constant space and be sufficiently fine and ponderous to overcome the varying densities of the solutions in

which it acts. Talc appears to satisfy these two demands, and is without any reactive influence upon the other constituents. By the use of this medium I have obtained the following results:—

100 cc. of various urines Purin N estimated by Camerer's method. (Kjeldahl N).	100 cc. urine Silver—magnesium—purin—talc precipitate.
0·0299	25 cc.
0·0287	24 „
0·0258	23 „
0·0153	13 „
0·0150	13 „
0·0162	15 „
0·0152	15 „
0·0174	$15\frac{7}{8}$ „
0·0162	15 „
0·0128	12 „
0·0130	12 „
0·0184	16 „
0·0168	$14\frac{1}{2}$ „
0·0124	12 „
0·0106	11 „
0·0221	21 „
0·0244	23 „
0·0163	15 „
0·0170	16 „
0·0259	24 „
0·0202	$18\frac{1}{2}$ „

From these figures it would appear that the precipitate maintains a somewhat constant relation to

the amount of N. found by Kjeldahl's method, and gives a factor of 0·0011 N. per cc. The table on page 147 states the results obtained by the use of this factor compared with those found by Camerer's method. Three separate estimations of 100cc. urine were made at the same time. Compared with a similar estimation by Camerer's method, the constancy of the quantity of the silver-purin-talc precipitate confirms the previous figures.

Table XXII.

Amount of Precipitate.

Subjects of experiment.	Measure I.	Measure II.	Measure III.	Measure IV. Silver ppt. withour talc.	Calculated N value	Camerer N.
Dr. K.	24cc.	23cc.	24 cc.	28cc.	0·0258	0·0244
Prof. S.	15 ,,	15 ,,	15 ,,	22 ,,	0·0165	0·0163
,, S.	15 ,,	16 ,,	15 ,,	—	0·0170	0·0170
Vaktmästare, J.	24 ,,	25 ,,	24 ,,	31 ,,	0·0269	0·0259
Patients from Serafimer lazarettet						
1. Pleurisy	19 ,,	18 ,,	19 ,,	31 ,,	0·0203	0·0202
2. Cardio-sclerosis ...	10 ,,	11 ,,	10 ,,	—	0·0115	0·0116
3. Polyneuritis	13 ,,	13 ,,	13 ,,	—	0·0143	0·0160
4. Acute nephritis ...	4 ,,	5 ,,	4 ,,	—	0·0049	0·0030
5. Chronic alcoholism	10 ,,	10 ,,	11½ ,,	—	0·0121	—
6. Cardiac disease	4 ,,	4¼ ,,	4 ,,	—	0·0046	0·0033

For the opportunity to examine these pathological urines I am indebted to the Physicians of the Serafimer Lazarett, Stockholm.

Expressed as total quantities of daily purin excretion, the results of the two methods show close approximation: —

	Quantity of urine	Measured ppt. N	Camerer N	In terms of uric acid and xanthin
Dr. K.	1000cc.	0·2580	0·2440	0·7320
Prof. S. ...	1250 ,,	0·2062	0·2047	0·6131
V. J.	1200 ,,	0·3228	0·3108	0·9324
Patient				
1	1150 ,,	0·2340	0·2323	0·6969
2	1500 ,,	0·1725	0·1730	0·5190
3	2000 ,,	0·2860	0·3200	0·9600
4	1800 ,,	0·0680	0·0540	0·1720
5	2000 ,,	0·2420	—	0·7260
6	1200 ,,	0·0430	0·0396	0·1290

Although I cannot assume that the factor 0·0011 N. per cc. of purin precipitate is absolutely correct, yet it will form a basis for the work of other observers, and lead, I hope, to the establishment of a definite factor. In the cases cited above, however, it gives very close results, and the figures agree most closely when the quantity of the urine approaches the normal. If the amount of urine and its specific gravity are markedly abnormal, the precipitate falls differently. The use of talc to a great extent overcomes this difficulty; concentrated urines may be easily diluted, and dilute urines may be evaporated to any desired quantity in slightly acid solution. As Camerer's process involves the use of ammonia to fully dissolve the silver-chloride formed, the question as to the varying amounts of sodium chloride in pathological urines is important, for in order to obtain a near uniformity of density, the amount of fluid must not exceed a certain number of cc. Additionally, any excess of ammonia interferes with the proper precipitation of the purin bodies. As to the silver chloride, I have in many instances added 20 drops of concentrated HCl to 60cc. of urine, and experienced no difficulty with the solution of the chlorides in NH_4OH. In a case of chronic alcoholism, however, I was unable to entirely dissolve the silver chloride, and the part remaining fell with the purin precipitate, increasing its amount. A proposal to overcome this difficulty will be advanced later. Albuminous urines must be freed from albumin by boiling in slightly acidified solution. Medium amounts of sugar do not affect the precipitation, but

with very large amounts, it is better to precipitate the urine with excess of copper sulphate and sodium bisulphite, decompose the precipitate by H_2S or K_2S, and then reprecipitate with ammoniacal silver nitrate solution.

The method I have used is as follows:—After noting the total daily quantity, the urine is tested for albumin. If present, this is removed by *slight* acidification with acetic acid and boiling. Two solutions are necessary:—

No. 1 solution.		No. 2 solution	
Ludwg's Magnesium Mixture	100 cc.	Silver nitrate - -	1 grm.
Ammonia solution 20 % -	100 cc.	Ammonia (strong) - -	100 cc.
Talc ..	10 grm.	Talc - - - :	5 grm.
		Distilled water -	100 cc.

To 90cc. of the urine, 20cc. of No. 1 solution are added. An immediate precipitate of the phosphates falls, and the clear fluid may be removed by filtration or decanted into another graduated measure glass. This precipitate should not be allowed to stand longer than 15—30 minutes, as otherwise the uric acid may be partially precipitated. To 80cc. of the filtrate 18cc. of solution No. 2 are added. The resultant precipitate is a mixture of silver-chloride and silver-purin; the former body is dissolved by the excess of ammonia. The filtrate should be shaken until all white flakes disappear and the finely granular yellow-white precipitate remains suspended. If the AgCl is not entirely dissolved, strong NH_4OH is added drop by drop and the solution well shaken until no AgCl remains. The measure-glass is then corked and placed in a cupboard or corner protected from strong light. After an hour the purin precipitate will have

entirely fallen, but it is best to wait 24 hours before reading the result. If the precipitate occupies 7cc., then this amount multiplied by 1·5 and 0·0011= percentage quantity of purin-N.=0·0100 per cent., and multiplied by the total daily amount of urine, say 1500cc.=0·1500 N. or in terms of uric acid and xanthin bases=0·45gm. Should it now be desired to estimate the precise total purin-N. by Kjeldahl's method, this may be easily performed after the precipitate has been washed with water (60°C.) until the filtrate is neutral to lacmus paper, or the exact amount of uric acid may be estimated in the precipitate by the Ludwig-Salkowski method. The process does not interfere with the details of the ordinary laboratory methods, but yields an approximate result which for clinical purposes is sufficiently accurate, and allows the estimation to be attained at little cost of time and apparatus. Still, two measure-glasses are necessary, and the trouble of preparing filter paper, and the process of filtration occupy a certain amount of time. It would be of advantage, therefore, to have an apparatus in which the whole process could be completed easily. I think that the "purinometer" I now propose will meet this requirement. It consists essentially of three parts:—

1. A closed, graduated tube.
2. A stop-cock, with a bore of the same diameter as the upper tube.
3. A small glass reservoir of known cubical capacity.

It is used in the following manner:—With the stop at a right angle to the tube, urine is poured in

up to 90cc. The stop-cock is then turned parallel with the tube, and the lower chamber and the bore of the tap become filled with the urine.; 20 cc. of solution No. 1 is then added and the precipitate allowed to settle. If the reagent contains no talc, the precipitated phosphates take a long time to settle; there is some loss of uric acid. To demonstrate the time saved by the addition of talc, an experiment may be cited. At 3-30 p.m. (Nov. 13), 60cc. urine, 7cc. Ludwig's magnesia mixture and 7cc. 20 per cent. ammonia solution were placed in two graduated measures. To one of these 5cc. of a 5 per cent. talc solution was added.

P.m.	With talc	Precipitate	Without talc	
3·35	phosphate precipitate	= 18 cc. ...	phosphate precipitate	= 72cc.
3·40	,, ,,	= 16 ,, ...	,, ,,	= 70 ,,
3·45	,, ,,	= 14 ,, ...	,, ,,	= 30 ,,
3·50	,, ,,	= 13 ,, ...	,, ,,	= 22 ,,
3·55	,, ,,	= 11½ ,, ...	,, ,,	= 17 ,,

The precipitate of phosphates sinks into the lower chamber of the purinometer, and immediately this has happened the tap is again turned at right angles. To the clear fluid now remaining in the upper tube, solution No. 2 is added to make the total fluid 100cc. The resultant precipitate consists of a mixture of silver-chloride and silver-purin. The apparatus is then inclined backwards and forwards until the precipitate is yellowish-white. This can be readily seen by comparison with the white phosphate precipitate in the lower tube. The instrument is now allowed to stand 24 hours, when the percentage of purin may be read off at the upper level of the precipitate. Varia-

tions in the quantities of phosphates can also be observed by a similar grading of the lower chamber. An advantage may be claimed in the case of an excess of chlorides, which solution No. 2, or even a few added drops of strong ammonia will not dissolve. The silver-chloride is heavy and falls rapidly (within a minute), whilst the lighter purin precipitate takes an hour or so to settle. It is possible, therefore, to allow the silver-chloride to fall, turn the stop-cock, let the ppt. pass into the bore of the tap, and immediately return the tap to a right angle. The loss of any purin-silver is by this means exceedingly light, and the silver-chloride excess does not interfere with the estimation. As the purinometer is graded in cc., it can be used for ordinary measuring purposes when occasion demands. The temperature of the room in which the estimations are made should be between 10°—15°C.

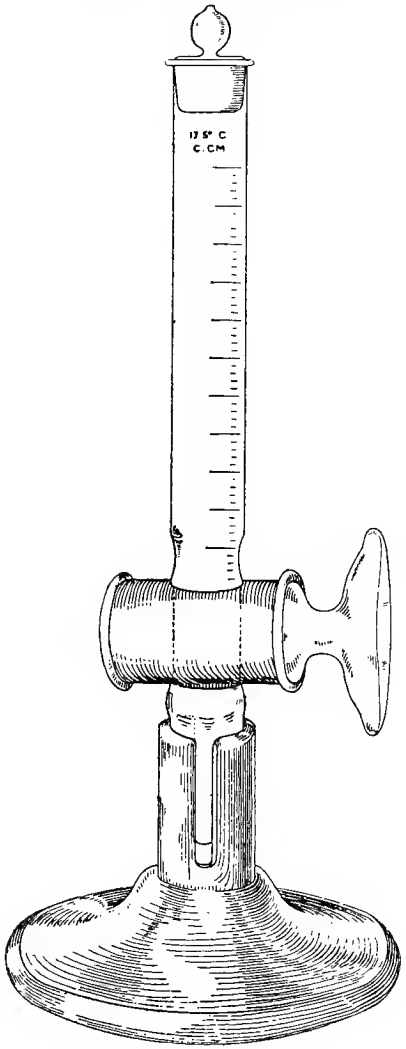
The instrument is also of use for the collection and examination of urinary sediments for pus, casts, crystals, etc. When the insoluble substances have fallen, they can be passed into the lower chamber by slightly turning the stop-cock, and then, after emptying the upper chamber, be transferred to an object-glass, or if tubercle bacilli are suspected, directly centrifugalised in an ordinary tube. In both cases work is facilitated.

By use of the centrifuge, the precipitates became constant in a few minutes, and there was no necessity for the addition of talc. Where such apparatus exists, the convenience of the method is increased

and the results are more regular. A graduated centrifugalising tube is however necessary.

Later experience shows that the results attained compare well with control estimations made by the Camerer process for total alloxuric bodies when the S.G. of the urine is between 1015 and 1025. With urines whose specific gravity is from 1000—1005, the percentages stated in the tables are too high, and for accurate estimations the urine must be evaporated in slightly acid solution to considerable concentration, although for clinical purposes the precipitates give relatively correct figures and allow useful comparison.

The purinometer has been made for me by Gœtze, of Leipzig, and may be obtained from Messrs. Gallenkamp, Sun Street, Finsbury.



THE PURINOMETER

CHAPTER XI.

SUMMARY.

OF the known purin-bodies, hypoxanthin, xanthin, guanin, and the methyl-xanthins, caffeine and theobromin are found in foodstuffs, and uric acid, and traces of xanthins and methyl-xanthins are met with in the urine.

1. As the methods available for the estimation of purin-bodies in animal organs were unsatisfactory, both as to technique and results, modifications were introduced and a reliable process worked out.

2. In foodstuffs, the purin-bodies occur in two forms, "*free*" and "*bound*." Both the glandular and muscular tissues contain approximately equal amounts of "*free*-purins," but the glandular tissues yield very large and the muscles only very small quantities of bound-purins (nucleins).

3. The estimations of the purin bodies contained in meats, show that considerable quantities are present, but that little difference exists between the amounts contained in white and dark meats.

4. Certain vegetable foods have been found to contain purin bodies. Amongst these are peas, beans, oatmeal, asparagus and onions. This furnishes a reason for the high uric acid excretion which follows their ingestion.

5. From several varieties of beer and porter purin-

bodies have been isolated, and their percentage amounts estimated. Their presence may account for the harmful influence of these beverages in gout, and for some of the pathological changes which occur in chronic alcoholism.

6. Experiments upon the action of the purin-bodies upon carbohydrate metabolism show that caffeine induces an increased elimination of CO_2. Uric acid and hypoxanthin, however, are inert in this regard.

7. The continued daily injection of hypoxanthin into rabbits hinders their growth, causes degenerative cell changes in the liver and kidneys, alters the cellular relations and contents of the blood and marrow, and produces slight changes in the intima of the smaller blood-vessels.

8. Feeding experiments with fish, fowl, beef, haricot beans and beer, under appropriate conditions, show that the urinary purin is increased in all these cases; that this increase corresponds with 50 to 60 per cent. of the purin-bodies ingested with the food; that the purin is principally in the form of uric acid, and that the increase of urinary purin reflects the metabolic activity of the individual in regard to nucleins.

9. The fæces may contain unabsorbed nucleins as well as certain purin substances from the digestive juices and cell-nuclei, and estimations of these bodies should be included in all metabolic experiments.

10. When the " free " purins are ingested, they are rapidly oxidised and decomposed. About 50—60 per cent. of hypoxanthin leaves the body as urinary purin

(principally uric acid) within 4—6 hours, and the same percentage of uric acid appears in the urine after 8—10 hours. The bound purins, however, take 1—2 days before they are fully excreted.

11. The remaining 50 per cent. of the food-purin is excreted as urea, or as bodies intermediate between uric acid and urea.

12. By the quantitative estimations of purin-bodies in foodstuffs, an exact forecast of the exogenous urinary purin is possible, and its amount can be limited when necessary by prescribing a certain diet. From the total urinary purin the exogenous portion can be deducted and the endogenous amount obtained. Tables iv., vii., and vii. should, therefore, not only be useful for dietetic purposes, but save a considerable amount of laboratory work.

13. The endogenous purin is partly derived from leucocytes, but mostly from the cell changes which result in the maintenance of bodily functions. Hence, as the cell-nucleus is the dominating factor in metabolism, the cleavage of cell nucleins may incite the decomposition of proteid matter. It is possible that the endogenous urinary purin represents about one-half of the total endogenous purin produced, and that the latter quantity indicates the extent of metabolic processes more completely than any other factors at present available.

14. Uric acid is a necessary result of normal nuclein metabolism. In disease it is symptomatic of conditions which hinder or prevent its solubility and excretion, and does not itself cause the lesions which accompany uricacidæmia. Drugs are unable to

increase its solubility in the bloodstream, but they may promote the normal processes of nuclein metabolism through hepatic stimulation. Uratic deposits take place slowly and their infiltration might perhaps be decreased by the administration of organic compounds which delay the precipitation of uric acid from its solution.

15. The general conclusions of the investigation point to the need for determinations of the *endogenous* purins in many diseases, either by the use of purin-free food, or by the aid of tables giving the percentages of purins in foodstuffs, and the necessary calculations therefrom. In order to assist the clinician in his attainment of such records, an instrument —the purinometer—is proposed, and the method for its use is described.

16. The action of the purin bodies upon the alimentary system, as demonstrated by Pawlow and later by Potapow-Procaitis, strongly contra-indicates the employment of meat extracts or soups in hyperchloridia.

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APPENDIX.

Constitution and Calorific Values of certain Foodstuffs.

	Water	Proteid	Fat	Carbo-hydrates	Purin bodies grms per kilo	Salts	Calories
Fish :							
Cod	76.1	23.	0.2	—	0.5	0.7	103.
Plaice	77.9	18.7	2.4	—	0.7	1.0	104.
Salmon	76.	15.	7.	—	1.1	2.0	132.
Halibut	75.2	19.9	3.9	—	1.0	1.0	124.
Meat :							
Beef	72.	21.	6.	—	1.3—2.0	1.1	133.
Fat	54.	16.	27.	—	1.1	1.	213.
Mutton	76.	18.	5.	—	0.96	1.	150.
Fat	48.	15.	36.	—	—	0.8	390.
Veal	76.	20.	6.	—	1.1	1.3	180.
Fat	72.	18.	8.	—	—	1.	220.
Pork	72.	19.	6.	—	1.2	1.1	146.
Fat	47.	14.	37.	—	0.5	0.7	406.
Ham	41.	23.	36.	—	1.1	1.0	434.
Meat Soups	—	8.	0.2	1.2	varying large amounts.	17.5	7.
Chicken	76.	19.5	1.5	—	1.2	1.3	100.
Vegetables :							
Potatoes	74.	2.	0.2	20.0	0.02	1.	92.
Rice	10.	6.7	0.8	78.5	—	1.	356.
Flour, white	16.5	13.	1.5	68.3	—	0.7	350.
Bread, white ...	40.	7.	0.5	55.4	—	1.3	260.
Oatmeal	15.	13.	6.	63.	0.53	3.0	177.
Peas	14.3	21.1	0.8	61.	0.39	2.6	344.
Lentils	12.5	24.8	1.8	58.4	0.38	2.5	343.
Beans (Haricot)	14.8	25.1	1.8	51.5	0.63	3.1	330.
Asparagus	93.3	1.9	0.2	2.3	0.21	0.5	18.7
Cabbage	80.0	3.9	0.9	10.4	—	1.5	64.
Lettuce	94.3	1.4	0.3	2.1	—	1.0	16.6

	Water	Proteid	Fat	Carbo-hydrates	Purin bodies grms per kilo	Salts	Calories
SPECIAL FOODS :							
Milk	88.5	3.4	3.6	4.8	—	0.7	64.
Butter	13.2	0.8	85.	—	—	1.0	793.
Eggs	75.6	13.0	11.0	—	—	1.4	155.
Cheese (fat)	37.8	27.2	30.0	2.5	—	1.5	404.
DRINKS :							
Beer, Lager	88.7	0.7	—	5.0	0.12	0.2	47.9
Ale	89.1	0.5	—	4.8	0.14	0.1	46.
Porter	88.0	0.6	—	5.0	0.15	0.3	47.
					per pint		
Tea	—	0.6	—	1.0	1.2	—	6.5
Cocoa	—	2.0	2.0	4.0	1.0	—	43.2
Chocolate	—	5.1	15.2	74.9	0.7	—	469.
Coffee	—	0.5	0.6	1.6	1.7	—	14.7
					Alcohol		
Claret	—	—	—	0.3	8.0	—	57.2
Sherry	—	—	—	1.5	17.1	—	125.8
Brandy	—	—	—	—	45.0	—	315.0

METHODS.

Estimation of purin bodies in meats and meat extracts see p. 33.

Estimation of purin bodies in vegetable foods see p. 37.

Preparation of Nuclein:—

Mince a thymus or pancreas gland thoroughly, or pass it several times through a sausage machine, and then macerate the pulp with four times its bulk of 0·5 per cent. solution of ammonia. Stir frequently. Strain through muslin, and similarly extract the residue several times. Mix the extracts together, and acidify with five per cent. HCl until a faintly acid reaction is obtained.

The brown or dirty white precipitate is nucleo-albumin. To prepare nuclein from this, add ten times its bulk of 0·2 per cent. HCl and 5—15cc. of liquor pepticus. Digest the mixture for 48 hours at 37°C upon a water bath or in an incubator. The albumin will have been split off as peptone, and the nuclein will be precipitated as a brown sediment. To purify it, filter, wash the residue with 0·2 per cent. hydrochloric acid, re-dissolve it in 0·5 per cent. ammonia solution, and re-precipitate it by the addition of five per cent. acetic acid. Repeat the process several times, and then weigh the purified nuclein. Boil an aliquot portion in 0·5 per cent. sulphuric acid for 12 hours and determine the quantity of purin-nitrogen as on p. 33.

Preparation of Nucleic Acid:—

The nuclein is decomposed by caustic soda, a sodium nucleate is formed, and the nucleic acid is freed by the addition of acid alcohol.

Place a weighed quantity of nuclein in 100cc. of a 3 per cent. caustic soda solution. Stir the mixture during 5—10 minutes, make it neutral with 0·5 per cent. hydrochloric acid and then add acetic acid to precipitate the proteid. Filter and make up the filtrate to 200cc. Add 5cc. of 20 per cent. hydrochloric acid and 200cc. of 0·4 per cent. acid alcohol (hydrochloric) and collect the precipitated nucleic acid upon a hardened filter paper. Wash the residue into a small beaker, re-dissolve it in 0·5 per cent. ammonia solution and re-precipitate the nucleic acid by the addition of acid alcohol. Dry at 90°C then over sulphuric acid and weigh.

Preparation of Nucleic Acid (after Neumann):—

Place one kilo of pancreas or thymus in boiling water for a few minutes and weakly acidify. Then mince thoroughly and heat for half-an-hour in two litres of water, 100cc. of 33 per cent. caustic soda solution, and 200grms. of sodium acetate. Then neutralise with 150cc. of 50 per cent. acetic acid, and filter whilst hot. Next at 40°C add an equal volume of alcohol, and allow the solution to cool. Filter and dissolve the residue in 500cc. of water, heating upon water bath until the solution is quite clear. Filter and precipitate the nucleic acid with acid alcohol. Purify by dissolving in weak ammonia solution and re-precipitating with 0·5 per cent. hydrochloric acid.

Preparation of Guanylic Acid (after Ivor Bang):—

Mince thoroughly one kilo of pancreas gland, and allow it to stand for 24 hours in one per cent. solution of sodium hydrate. Warm until the mixture pours easily, neutralise with acetic acid and then make it distinctly acid. Filter and boil the residue several times with water. Add all the filtrates together—they should yield 5—6 litres—filter again, make alkaline with 0·5 per cent. ammonia solution and evaporate to 300cc. Warm, add 900cc. alcohol, allow to stand for four hours and then filter. Dissolve the residue in 150cc. of hot water and filter whilst hot. Boil the remainder several times, filter, add three volumes of alcohol to the filtrate, cool and filter. Wash the precipitate of guanylic acid with alcohol, alcohol and ether, and ether, dry over sulphuric acid and weigh.

Estimation of Phosphorus in Nucleins:—

Fuse the substance in a platinum or silver capsule with a little caustic potash and potassium nitrate. This liberates the phosphorus from the organic matter, and it combines to form potassium phosphate. When cool, dissolve the mass in water, and acidify with nitric acid. Precipitate the phosphorus by adding about one-fourth of its bulk of a solution of ammonium molybdate containing nitric acid and a similar quantity of a saturated solution of ammonium nitrate. Place the mixture on a water bath at 50°C., collect the yellow precipitate of molybdium phosphate on a hardened filter paper, wash it with ammonium nitrate solution, and then dissolve it in 3 per cent. ammonia solution. Add some Ludwig's magnesia mixture, filter after 24 hours on a Schliecher and Schull's No. 575 ash free filter paper, and incinerate. The ash, which consists of $Mg_2P_2O_7$, may be weighed, and its phosphorus calculated.

Estimation of "Carbohydrate" in Nucleins:—

Weigh about 0·2gm. of the dried nuclein, add to it 100cc. of 20 per cent. sulphuric acid, heat on a water bath at 60°C. for four hours. Filter, wash the residue with distilled water, titrate the filtrate with Fehling's solution, and calculate the inverted sugar in terms of dextrose. For further differentiation of xylose, pentose, etc., consult *Grund* ("Zeit. f Physiolog. Chemie.," Bd. 35) or *Bang* ("Hoffmeister's Beiträge," s. 175, 1903).

Estimation of Total Nitrogen (Kjeldahe):—

Incineration. Add 5—30cc. of concentrated pure sulphuric acid to about 0·5gm. of organic matter or 10cc. of urine. Heat the mixture in a hardened Jena flask of about 300cc. capacity until it is quite clear and white in colour. This part of the process may be hastened by adding a few crystals of copper sulphate and about 3 grams of potassium sulphate or potassium pyrophosphate. When the solution becomes light yellow in colour, the carbon has been nearly all oxidised to CO_2 and the nitrogen changed into ammonia at first, and later into ammonium sulphate. A few crystals of pure potassium permanganate may be added to hasten the final stages. Except when filter papers or considerable quantities of organic matter require incineration, it is best to use sulphuric acid and the crystal of copper sulphate only, even if the process lasts over twelve hours.

Distillation. Allow the contents of the combustion flask to cool. Meanwhile measure 10—50cc. of deci-normal sulphuric acid solution into a small Erlenmeyer's flask, add a few drops of neutral lacmus or alizarin solution, and connect the flask with the distillation apparatus, so that the block-tin tube just touches the fluid. When the incineration solution is quite cold wash it into a hardened glass distillation flask of about 1000cc. capacity, and add about 10—20 grams of talc to prevent bumping. Talc is more satisfactory than zinc or glass tubing. Pour down the side of the flask a sufficient quantity of 33 per cent. caustic soda to make the mixture thoroughly alkaline. The reaction may usually be taken rapidly with litmus paper without any loss of ammonia. Attach the flask to the distillation apparatus, heat at first

It has been lately pointed out that the addition of solid potassium permanganate frequently leads to loss of nitrogen, so that it should not be used when accurate estimations are desired.

slightly, then strongly, and turn on the water at slow speed. Continue the distillation until the drops of distillate as they appear react neutral to litmus paper or to a drop of neutral lacmus or alizarin allowed to run down the tin tube so as to mix with the drop as it appears.

Titration. It is more satisfactory to use a decinormal solution of barium hydrate than one of sodium hydrate. Run the alkaline solution into the distillate until the lacmus turns blue or the alizarine turns yellow. Then deduct the number of cc. of alkali used from the number of cc. of acid taken and multiply the factor obtained by 0·0014. The figures obtained will give the amount of nitrogen in grammes contained in the substance taken.

Estimation of Urea (Mörner and Sjöqvist):—

Solutions required.

1. Saturated solution of barium chloride (with 5 per cent. baryta).

2. A mixture of 1 vol. ether and 2 vols. absolute alcohol (preferably in a wash-bottle).

Take 5cc. of urine, 5cc. of barium mixture, 100cc. of the alcohol-ether mixture in a small stoppered flask and allow to stand for twelve hours. Filter and wash the precipitate with the alcohol-ether. Remove the alcohol and ether from the filtrate by evaporation at 55°C. (not above 60°C.). When the liquid is reduced to 25cc. add a little magnesium oxide and about 15cc. of distilled water. Continue the evaporation, and when the ammonia is entirely removed and the fumes

are no longer alkaline in reaction add a few drops of concentrated pure sulphuric acid. Now transfer the liquid to a Kjeldahl combustion flask, evaporate a little further, add 20cc. of strong sulphuric acid and estimate the nitrogen contents by Kjeldahl's process. To state the nitrogen in terms of urea multiply by 2·143.

Estimation of Ammonia (Schlosing's method):—

Take 25cc. of urine and place it in a flat glass dish with vertical sides, and mix it with 20cc. milk of lime. Into another similar vessel place 20cc. of deci-normal sulphuric acid. Cover both with a bell jar and allow to stand for several days. The sulphuric acid absorbs the ammonia after its expulsion by the lime, and the amounts of acid which has not been neutralised is determined by titration. This method gives rather low results.

Estimation of Ammonia by the Vacuum Method after Schittenhelm ("Zeit f. Physiolog. Chemie.," Bd. 39):—

To 25—50cc. of urine (or equal quantity of solid tissues) add 10gms. sodium chloride and 1 gm. sodium carbonate. Place the flask in a water-bath and distil through iced water into 10—30cc. decinormal acid containing a few drops of rosolic acid. Produce a vacuum in the receiving flask and keep the water-bath at a definite temperature of 43°C. Replace the evaporated fluid in the distilling flask by a few cc. of water or alcohol run in through a separator funnel. The entire process should occupy 30—40 minutes. Carefully disconnect the vacuum flask, allowing air to enter slowly,

and titrate the distillate with deci-normal caustic soda or barium hydrate.

Ex. 30cc. urine + 10gms. NaCl + 1gm. Na_2CO_3 + 20cc. C_2H_5OH after twenty-five minutes at 43°C. neutralised 7·03cc. deci-normal acid. This quantity equals 0·0119 NH_3.

Estimation of the Total Purin Bodies (Camerer-Arnstein).
Solutions required.

1. Magnesia mixture.

Magnesium chloride (crystal) ...	110gms.
Ammonium chloride	110 „
Ammonia	250 „
Water to	1000cc.

2. Ammoniacal silver nitrate.

Silver nitrate	26gms.
Water	300cc.
Add ammonia until the silver oxide re-dissolves and dilute the solution to	1000cc.

3. Ammonia solution (20 per cent.).

To 240cc. of urine, from which all proteids have been removed by acidification and boiling, add 30cc. of magnesia mixture and 20cc. of the 20 per cent. ammonia solution. Allow to stand for 15 minutes and then filter. Take of the filtrate two portions of 125cc. each—125cc. corresponds to 100cc. of urine. To each add 10cc. of ammoniacal silver nitrate, and after a few minutes filter through an ash free filter paper of about 10cm. diameter. Wash out the vessel used for precipitation, with weak ammonia solution, and the precipitate with distilled

water at 60°C until the washings are no longer alkaline. Transfer the filter paper and the precipitate to a Kjeldahl's combustion flask, add about 0·5gm. of magnesium oxide and boil the solution almost to dryness. Then add 20cc. concentrated pure sulphuric acid and a crystal of copper sulphate and determine the nitrogen contents by Kjeldahl's process.

Modification by Purinometer. See page 149.

Estimation of Uric Acid (tri-oxy-purin) (Ludwig-Salkowski):—

Solutions required.

1. Magnesia mixture.

Magnesium chloride	... ...	110gms.
Ammonium chloride	... ...	110 „
Ammonia	... ...	250 „
Water to	... ...	1000cc.

2. Silver nitrate solution ... ... 3p.c.
3. Sodium sulphide solution.

   10gms. pure caustic soda dissolved in 1000cc. of water. Divide into equal parts; saturate one with sulphuretted hydrogen and then mix the two together.

To 200cc. of urine add 50cc. magnesia mixture and 50cc. 20 per cent. ammonia solution. Filter, take 200cc. and add 10—15 cc. of 3 per cent. silver nitrate solution. Wash the precipitate with weak ammonia solution and then with water, puncture the filter and wash the precipitate into another beaker. Heat to boiling

10—20cc. of the sodium sulphide solution, and allow it to flow through the filter into the vessel containing the silver precipitate and then warm the contents on a water bath for 30 minutes, stirring constantly until the precipitate is entirely dark brown and no light specks are visible. Filter, add a few drops of concentrated hydrochloric acid, evaporate to about 15cc., add a few more drops of hydrochloric acid and allow to stand for 24 hours. The uric acid, which has crystallised out, should be transferred to a small paper, or, better, a glass wool filter, washed with a few cc. of water, alcohol, ether and carbon disulphide, and dried for four hours at 100°C. It may then be weighed, or its nitrogen determined by Kjeldahl's process. The amount of nitrogen found, multiplied by three, will give the quantity of uric acid in grammes in 133·3cc. For each 10cc. of the watery filtrate add 0·00048gms. uric acid to the quantity found directly.

Hopkins' method.

Solutions required.

1. Pure ammonium chloride
   (free from iron and entirely soluble in water).
2. Pure ammonium sulphate.
3. Potassium permanganate solution
   Potassium permanganate ... ... 1·578gm.
   Water ... ... 1 litre.

Add 30gms. of ammonium chloride to 100cc. of urine and shake until the salt is dissolved. Now add 1cc. of strong ammonia. Plug the flask with cotton wool and

allow it to stand until the ammonium urate has settled and the upper layer of liquid is quite clear. This stage requires from one to two hours. Filter and wash out the vessel with a saturated solution of ammonium chloride, and also wash the precipitate with the same solution. Transfer the precipitate from the filter to a beaker by a jet of hot water. Not more than 30cc. of water should be used. If more has been taken, add 1cc. of strong hydrochloric acid, concentrate on a water bath and then heat the solution to 90°C. Allow the uric acid to crystallise out and collect it on a hardened paper or glass wool filter, wash it with a few cc. of cold distilled water, alcohol and ether and weigh it.

Instead of weighing, the first precipitate may be washed with a saturated solution of ammonium sulphate, transferred to a beaker, water added to 100cc. 15cc. concentrated sulphuric acid are then run in and the mixture at once titrated with the permanganate solution. The end point is reached when the permanganate gives to the fluid a diffused pink flush. The colouration should be apparent throughout the solution, but rapidly disappears. The amount of cc. of permanganate solution used, multiplied by 0·00375, will give the amount of uric acid in grammes contained in the 100cc. of urine.

Dimmock and Branson's modification, see p. 142.

Estimation of the Xanthin or Purin Bases:—
Solutions required.

1. Magnesia mixture (see total purins).
2. Silver nitrate solution (3 per cent.).

3. Potassium or sodium sulphide solution.

Caustic potash 15 grammes.
Water..... 1000cc.

Divide solution into equal parts, saturate one with sulphuretted hydrogen; then mix the two together.

To 500cc. of urine add 50cc. of magnesia mixture. Allow the phosphate to settle, then filter and take 400cc. of the filtrate for the estimation. Add 30cc. of the silver nitrate solution and a few cc. of strong ammonia solution, and filter after 1—2 hours. Wash the beaker and the precipitate with weak ammonia solution and with distilled water at 60°C. Then transfer the precipitate to another beaker, decompose it with 30cc. of the potassium sulphide solution or by H_2S. Filter, acidify the filtrate with hydrochloric acid and then evaporate the solution to 10cc. on a water-bath. Allow the fluid to stand for twelve hours, so that the uric acid may crystallize out. Remove the uric acid by filtration, make the filtrate thoroughly alkaline with ammonia and precipitate the purin bases by adding silver nitrate solution. Wash the purin-silver precipitate and the beaker, first with weak ammonia solution, then with distilled water at 60°C., until the washings are no longer alkaline. Then transfer the filter and precipitate to a combustion flask, add a little magnesium oxide and 50cc. of water, boil down to 10cc. and then determine the nitrogen contents by Kjeldahl's process. One gramme of nitrogen multiplied by 2·625 gives the amount of xanthin or purin bases present.

Or, the precipitate may be collected on a chlorine free filter, washed, incinerated, the ash dissolved in nitric acid

and titrated with ammonium sulpho-cyanide (1gm. silver corresponds to 0·277gm. nitrogen or 0·738 purin bases).

Estimation of Allantoin:—

1. After Lœwi.

Solutions required.

1. Solution of mercurous nitrate (containing a little metallic mercury to prevent further oxidation).

2. Silver nitrate solution (3 per cent.).

Add to 100—500cc. of urine excess of mercurous nitrate, filter, wash and saturate the filtrate with sulphuretted hydrogen. Remove the latter by evaporation, then add magnesium oxide and silver nitrate solution. Filter, wash with water, until the filtrate does not give a cloud with hydrochloric acid. Then determine the nitrogen by Kjeldahl's process.

2. After Poduschka.

Solutions required.

1. Solution of basic lead acetate.
2. Solution of sodium sulphate.
3. Solution of silver nitrate.

To 50—100cc. of urine add an equal volume of basic lead acetate solution. Take an aliquot portion of the filtrate, add sodium sulphate solution to remove the overplus lead. Filter, add 20—30cc. of the silver nitrate solution and again filter. The filtrate should not give any precipitate on the addition of silver nitrate. Add to it a few drops of very weak ammonia solution and a large volume of silver nitrate solution. Allantoin is precipitated as white or greyish-white flocculent masses. Filter, wash with a 1 per cent. solution of sodium sulphate

(perfectly free from ammonia), then estimate the nitrogen of the precipitate by Kjeldahl's process—1cc. $\frac{n}{10}$ sulphuric acid = 0·0039gm. allantoin.

Estimation of Chlorides.

Solutions required.

1. Silver nitrate solution.

Fused silver nitrate	29·075gms.
Water	1000cc.

1cc. = 0·01gm. sodium chloride.

2. Potassium chromate (neutral). Saturated solution.

Place 10cc. of urine and 90cc. of distilled water in a porcelain capsule, and add a few drops of the chromate solution until a distinct yellow colour is obtained. Take 25cc. of the silver nitrate solution in a burette, and, while stirring the diluted urine, run in the silver solution until an orange tint appears and remains after further agitation of the fluid. Deduct 1cc. for the other urinary substances that combine with silver nitrate and multiply the number of cc. used by 0·01. This gives the quantity of chlorides in 10cc. of urine.

Estimation of Phosphates:—

Solutions required.

1. Uranium nitrate solution.

Uranium nitrate	35·5gm.
Water	1000cc.

2. Sodium acetate.

Sodium acetate	100gm.
Glacial acetic acid	100cc.
Water	900cc.

3. Tinctura cocci B.P.

Total phosphates. Take 50cc. of urine in a small flask and add 5cc. of the sodium acetate solution and sufficient cochineal to colour the mixture a decided red. Fill a burette with the uranium nitrate solution. Boil the urine and run in the uranium until a faint green colour appears and is uniformly diffused. Again bring the mixture to the boiling point, and if the green colour is not permanent continue to add the uranium. Multiply the number of cc. used by 0.005 to obtain the quantity of P_2O_5 in 50cc. of urine.

Earthy phosphates. Add strong ammonia solution to 200cc. of urine till it reacts strongly alkaline. Allow to stand for 12 hours. Collect the precipitate of earthy phosphates on a hardened filter, wash with dilute ammonia solution, remove from the filter paper to a porcelain basin, dissolve the phosphates by adding a few drops of acetic acid, warming if necessary, make up the mixture with distilled water to 50cc., add 5cc. of sodium acetate and run in the uranium nitrate solution.

Alkaline phosphates. Subtract the amount of earthy phosphates from that of the total phosphates in 200cc. of urine and multiply the total daily urine $\frac{\text{number of cc.}}{200}$ by the factor resulting to obtain the amount of total alkaline phosphates.

Estimation of Sulphates:—

Solutions required.

1. Barium chloride solution.
2. Pure hydrochloric acid.
3. Pure sulphuric acid.

Total sulphates. Add 5cc. of concentrated pure hydrochloric acid to 100cc. of urine, and boil in order to decompose the aromatic sulphates. Then add barium chloride solution until no more precipitate is produced. Collect the barium sulphate on a hardened ash free filter paper, wash with boiling distilled water until the washings no longer give a precipitate with sulphuric acid. Dry the filter paper and residue from the washings as well as the precipitate in an oven at 100°C., weigh a crucible, transfer the dried sulphates, incinerate, cool over sulphuric acid, add a few drops of sulphuric acid, carefully heat to redness, again cool in a dessicator, weigh and deduct the weight of the capsule. This gives the quantity of barium sulphate (1 part of barium sulphate corresponds to 0·3433 parts of sulphuric acid).

Ethereal or aromatic sulphates. To 100cc. of urine add 100cc. of barium chloride solution. Filter very thoroughly through hardened filter paper. Take 100cc. of the clear filtrate (corresponding to 50cc. of urine), add 5cc. of hydrochloric acid and heat just above boiling point for five minutes. Then add barium chloride solution until no more barium sulphate falls, collect the precipitate on an ash-free hardened filter, wash well with boiling distilled water, and dry the residue in an air-oven at 100°C. Weigh a capsule, transfer the precipitate, heat to redness, allow to cool and add a few drops of pure sulphuric acid, then re-heat carefully and weigh. Multiply the amount of barium sulphate by 0·3433 to obtain the quantity of aromatic sulphates.

It has been suggested that instead of gravimetric estimations the barium sulphate precipitate formed after the addition of hydrochloric acid and barium chloride in

Modrakowski (*Zeit. f. Phys. Chemie.*, 38, 1903) takes 2gm. sodium peroxide in a nickel dish, adds slowly 50cc. of urine, evaporates to a syrupy consistence, heats the mass with spirit lamp until fusion occurs, then dissolves mass in water, filters, acidifies filtrate with HCl and then precipitates as usual with $BaCl_2$.

each case, should be centrifugalised in a graduated tube and the amount of the precipitate recorded. This method gives very good results in regard to the relations existant between the aromatic and total sulphates.

Estimation of Oxalates (after Autenrieth and Barth. "Zeit. f. Physiolog. Chemie.," Bd. 35):—

Solutions required.

1. Calcium chloride solution (saturated).
2. Ammonia solution (strong).
3. Hydrochloric acid, 15 per cent.
4. Ether-alcohol.

Ether	97cc.
Absolute alcohol ...	3cc.

Take 500—1000cc. of urine, add excess of calcium chloride and ammonia solution until the reaction is strongly alkaline. Shake thoroughly, and allow to stand twenty-four hours. Filter, dissolve the precipitate in 15—30cc. of 15 per cent. hydrochloric acid and then shake out the solution four or five times with 150—200cc. of the ether-alcohol mixture. Decant, allow to stand for an hour, remove the few drops of watery fluid remaining at the bottom of the vessel by passing the liquid through a dry filter and then distil the ether-alcohol extract. Evaporate the residue to about 5cc., add calcium chloride and ammonia to alkaline reaction, allow to stand for an hour and then precipitate the oxalate by adding weak acetic acid. After twenty-four hours collect the precipitate on a hardened filter of known

weight, incinerate in a previously weighed capsule and then determine the weight of the calcium oxalate.

Table of Measures.

Solids (approximates).

$^1/_{500}$	grain	= 0·00013	gramme.
$^1/_{100}$	,,	= 0·00065	,,
$^1/_{50}$	,,	= 0·0013	,,
$^1/_{40}$	,,	= 0·0016	,,
$^1/_{10}$	,,	= 0·0065	,,
1	,,	= 0·065	,,
5	grains	= 0·33	,,
10	,,	= 0·66	,,
15·4	,,	= 1	,,
$\frac{1}{4}$	ounce	= 7·1	grammes.
$\frac{1}{2}$	,,	= 14·2	,,
1	,,	= 28	,,
$\frac{1}{4}$	pound	= 112	,,
$\frac{1}{2}$	,,	= 225	,,
1	,,	= 450	,,
1 milligramme		= $^1/_{65}$	grain.
1 centigramme		= $\frac{1}{6}$	,,
1 decigramme		= 1·5	,,
1 gramme		= 15·4	,,
10 grammes		= $\frac{1}{3}$	ounce.
28	,,	= 1	,,
1 hectogramme (100gms.)		= $3\frac{1}{2}$	ounces.
1 kilogramme		= 2·35	pounds.

Liquids (approximates).

1 minim	= 0·06cc.
1 drachm (fluid)	= 4cc.
1 ounce „	= 30cc.
1 pint „	= 568cc.
½ gallon „	= 2·25 litres.
1 „ „	= 4·5 „
1cc.	= 16 minims.
4cc.	= 1 drachm (fluid)
30cc.	= 1 ounce „
100cc.	= 3½ ounces „

ATOMIC WEIGHTS.

Aluminium	27·1
Antimony	120
Arsenic	75
Barium	137·4
Bismuth	208·5
Lead	206·9
Borium	11
Bromine	80
Cadmium	112·2
Carbon	12
Cerium	140
Chlorine	35·5
Chromium	52·1
Cobalt	59
Copper	63·5
Erbium	166
Fluorine	19
Gallium	70
Germanium	72

Gold	197·2
Hydrogen	1·01
Indium	114
Iodine	126
Iridium	193
Iron	56
Lithium	7
Magnesium	24·3
Manganese	55
Mercury	200·3
Molybdium	96
Nitrogen	14
Nickel	58·5
Osmium	191
Oxygen	16
Palladium	106
Phosphorus	31
Platinum	194·5
Potassium	39·1
Radium	
Selenium	79·1
Silica	28·4
Silver	107·9
Sodium	23
Strontium	87·6
Tellurium	128
Thallium	204·1
Tin	118·5
Tungstium	184
Uranium	239·5
Zinc	65

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